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Unexplored Aspects of the Biotic Filter to Seedling Recruitment in Aquatic Environments

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UNEXPLORED ASPECTS OF THE BIOTIC FILTER TO SEEDLING RECRUITMENT IN AQUATIC ENVIRONMENTS

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DISSERTATION ABSTRACT

Sexual reproduction provides submerged aquatic vegetation (SAV) populations unique opportunities for dispersal, genetic mixing, and resilience in the event of catastrophic population declines. Relative to asexual reproduction, sexual reproduction is a risky resource investment and can have a lower probability of success. A wide variety of abiotic and biotic interactions common in both terrestrial and aquatic environments can lead to significant mortality of seeds and seedlings. The goal of this dissertation is to explore the diversity of biological interactions that influence seed and seedling survival in SAV that drive the population dynamics and restoration success of SAV species.

A combination of survey and experimental methods were used to test if three biological interactions, disturbance, herbivory, and competition, compromised seedling recruitment for three different SAV species growing in three different coastal environments. Chapter One explored the influence of sediment bioturbators on seedling establishment for the seagrass *Posidonia australis* in a marine environment. Field surveys demonstrated that dispersed seeds of *P. australis* overlap with a suite of sediment bioturbators that disturb the sediment in the coastal lagoons of Western Australia. The movement of sediment bioturbators found in these areas (sand dollars, sea stars, and heart urchins) dislodged and moved recently settled *P. australis* seeds. The overlap in habitat suitability between these animals and *P. australis* seeds suggests high densities of these animals consistently disturb non-dormant *P. australis* seeds and may act as a bottleneck to seedling recruitment. Chapter Two explored the role of grazers on seedling recruitment of a freshwater angiosperm. Field surveys recorded high grazing levels of isolated *Vallisneria americana* propagules in oligohaline areas of the James and Chickahominy Rivers, VA. Camera surveys identified the blue crab, *Callinectes sapidus*, as a likely herbivore. Subsequent surveys and experiments suggested *C. sapidus* in this system consumes SAV as part of their diet. The emergence of non-native SAV in the system, but not *V. americana,* suggests grazing prevents the recruitment of some SAV, but not others. Chapter Three evaluated interactions between adult plants and seedlings and how they impacted seedling establishment of an estuarine seagrass species. Field surveys consistently recorded seedlings establishing among existing *Zostera marina* shoots in a meso/polyhaline region of Chesapeake Bay, VA. Concurrent experiments indicated that seed supply influenced seedling establishment rates in some areas. Further surveys and experiments showed that negative interactions between seedlings and adult shoots influenced the subsequent survival of these seedlings within existing *Z. marina* meadows. Results from the research studies in this dissertation, conducted across three diverse coastal habitats, demonstrate that interactions between SAV seeds or seedlings and other biota can be very important in ultimately determining seed or seedling survival.

The diverse mechanisms through which biota compromise seedling recruitment and sexual reproduction for SAV observed here suggest there may be many additional, unexplored biological interactions affecting successful sexual recruitment for many SAV species. Because sexual reproduction provides substantial benefits to SAV populations, incorporating risks associated with seedling recruitment into population models and restoration strategies may help better predict SAV population health, resiliency and expansion as well as help optimize SAV restoration efforts.

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INTRODUCTION

Background

Clonal plants are angiosperms capable of both asexual reproduction, to form genetically identical ramets, and sexual reproduction, to form genetically distinct genets. Seeds, the product of sexual reproduction in flowering plants, are important vectors for evolutionary resilience not only for introducing genetic diversity to populations, but also dispersing progeny away from the ramets of the parent plant. Dispersal thus limits both competition with the parent plant and allows progeny to colonize remote habitats. Seeds can also serve as important insurance for populations to increase population resiliency after diebacks. If seeds exhibit a period of dormancy during stressful environmental conditions, they may more quickly repopulate gaps that emerged in parent populations than possible through asexual ramet production alone. Sexual reproduction and seeds can thus provide distinct benefits to clonal plant populations.

Seeds are, however, vulnerable and experience high mortality rates. Harper (1977) described a "sieve" of abiotic and biotic interactions limiting seedling recruitment in plant populations. Studies in terrestrial systems have linked abiotic factors, such as microsite or microhabitat availability (Eriksson & Ehrlen 1992), drought (Moles & Westoby 2004), and other environmental factors such as fire or rain scour (Leck & Outred 2008) to limits in seedling recruitment. Biotic interactions such as inter and intraspecific competition (Eriksson 1989, Eriksson 1993), trampling or physical damage (Moles & Westoby 2004), and faunal seed predators and herbivores (Janzen 1971, Wenny 2000) have also been identified to influence seedling survival. In terrestrial environments, a long tradition of research has explored many of the diverse abiotic and biotic interactions that can generate bottlenecks to seedling survival.

Research in aquatic environments suggests many of these same bottlenecks may affect seedling recruitment in submerged aquatic vegetation (SAV) (Kendrick et al. 2017). Abiotic factors, such as insufficient light, can have similar impacts on seedlings in aquatic and terrestrial environments (Bintz & Nixon 2001, Rivers et al. 2011, Zhang et al. 2014). Physical stresses in aquatic and terrestrial environments, however, may be quite different, as strong waves or tidal currents may uproot seeds and young seedlings (Valdermarsen et al. 2010, Marion & Orth 2012, Alagna et al. 2013, Statton et al. 2017). Biotic interactions can also impact seedling survival in aquatic as in terrestrial environments. Studies in aquatic environments have documented numerous instances of direct predation of seeds (Fishman & Orth 1996, Holbrook et al. 2000, Orth et al. 2002, 2007; Darnell & Dunton 2015, Statton et al. 2017). Additionally, because most SAV species are clonal, intra-, as well as, inter-specific competition have been hypothesized to limit seedling recruitment within existing meadows of SAV (Olesen & Sand-Jensen 1994; Yang et al. 2016). Despite results demonstrating the relevance of biota to SAV seedling recruitment, many biological and ecological phenomena potentially threatening SAV seedling survival, and ultimately sexual reproduction, are untested or undocumented. Given the importance of sexual reproduction on the genetic makeup and population dynamics of SAV, understanding the breadth and diversity of biological interactions impacting seedling recruitment is important.

Objective

The goal of my dissertation is to further the understanding of the diversity and breadth of biological interactions limiting seedling recruitment across a broad range of

natural and restored coastal SAV meadows. The experiments and surveys conducted in this dissertation measure the impacts of three biological interactions, disturbance, herbivory, and competition, on seed settlement and seedling recruitment for three SAV species in three different coastal environments. Results from these studies will show the breadth and diversity of biological agents and interactions affecting seedling mortality. By further quantifying the factors compromising seedling recruitment, this information may improve the ability of resource managers and scientists to better understand, model, and predict SAV population dynamics. This information may also allow resource managers and practitioners to optimize seed based mitigation and restoration strategies in coastal environments and to explain restoration failures.

The three chapters in my dissertation explore: 1. The impact of sediment bioturbators on seedling recruitment for the marine seagrass *Posidonia australis* in Western Australia; 2. The impact of an estuarine omnivore, *Callinectes sapidus*, on seedling recruitment of a freshwater SAV species, *Vallisneria americana*, within tidal freshwater and oligohaline regions of the Chesapeake Bay; 3. The impact of intraspecific competition on seedling recruitment of the seagrass *Zostera marina* in meso and polyhaline regions of the Chesapeake Bay. Each chapter highlights unique ecological interactions and biological agents impacting seedling recruitment for each SAV species in its environment. Together these three chapters evaluate the impact of biota on seedling recruitment in the biological and ecological context of each SAV species, and as such demonstrate the wide variability and strength of biotic interactions on seedling survival across a broad range of SAV community types and coastal environments.

Chapter Summaries and Implications

Chapter One

Chapter One of this dissertation explores the impact of a select group of sediment bioturbators on the seedling establishment of a temperate seagrass species in Western Australia. *Posidonia australis* is a long-lived and slow growing seagrass that produces abundant seed annually. Because *P. australis* clones grow densely, seedling recruitment is most likely to occur in bare sediment away from adult clones. Sediment bioturbators, such as heart urchins, sand dollars, and sea stars, scour bare sediments in search of food throughout much of the coastal waters of Western Australia. The pushing and burrowing of bioturbators in sediments onto which *P. australis* seeds settle could dislodge previously "safe" seeds from the sediment or bury seeds beneath critical depth thresholds. Because *P. australis* seeds have no dormancy and begin growing immediately upon release, they have a limited time period over which they must be incorporated in the sediment and establish root anchors. Newly produced leaf material may make seeds positively buoyant in as little as 2 weeks (*personal observation*). Statton et al. (2017) recorded 100% loss of seeds within one month in sheltered or moderately wave exposed environments with bioturbators present.

As a result, surveys were initially conducted to identify the potential overlap of sediment bioturbator communities and *P. australis* seeds at multiple natural and restoration sites at two locations in Western Australia. Experiments then tested the capacity of three observed sediment bioturbators to push and/or uproot recently settled seeds and one-year-old seedlings. The spatial and temporal overlap between seedlings

and sediment bioturbators and the capacity for these bioturbators to dislodge seeds found in this study suggests biotubators could be important bottlenecks to *P. australis* seed settlement and survival. Sediment bioturbator disturbance could prevent incorporation of seeds into "safe," buried environments or uproot recently buried seeds from this environment, resulting in displacement of seeds back into the water column. The rapid growth and buoyancy of displaced seeds, potential for strong wave energy (Statton et al. 2017), and the documented presence of seed predators in the region (Orth et al. 2002, 2006, 2007) makes displacement a severe threat to seed survival.

Chapter Two

Chapter Two investigates the impact of herbivory on SAV seedling and transplant survival within oligohaline regions of the Chesapeake Bay. Historically, *Vallisneria americana* was abundant in tidal, freshwater and oligohaline reaches of the James and Chickahominy Rivers, VA. Deteriorating water quality in the mid-20th century, however, decimated *V. americana* populations in this region. Populations of *V. americana* here have not recovered to their historic distribution despite improved water quality and long-term restoration efforts. Several non-native SAV species have, however, recently appeared and rapidly expanded in many places within the region (Moore et al. 2000, Moore et al 2010). Previous experiments protecting *V. americana* transplants and seedlings within mesh enclosures revealed a sub-aquatic herbivore was likely primarily responsible for these failed restoration attempts (Moore et al. 2010). This same herbivore is likely to have also prevented any potential recruitment of *V. americana* propagules

from nearby upstream populations where it continues to flourish into tidal areas immediately downstream.

An *in-situ* camera survey using adult *V. americana* transplants was used to first identify the potential herbivore community found within both the tidal James and Chickahominy Rivers. A previous study in the system suggests herbivory of adults and seedlings is the same and that the grazing of transplants could serve as proxies for the grazing of seedlings (Moore et al. 2010). Grazing intensity surveys were simultaneously conducted at both locations to estimate the rate over which *V. americana* propagules were consumed. Subsequent *in situ* experiments were then used to enclose blue crabs, *Callinectes sapidus,* the only herbivore observed in the camera survey, with *V. americana* transplants to assess differences in grazing between *V. americana* caged with or without *C. sapidus*. Laboratory experiments then tested if *C. sapidus* also ingest a non-native plant, *Hydrilla verticillata*, a species currently present in both the James and Chickahominy Rivers, at a similar rate to *V. americana*. Last, *C. sapidus* were collected outside experimental areas in the James and Chickahominy Rivers and were dissected to determine if *C. sapidus* in the system consume SAV for some portion of their diet. Results from this study demonstrate that an iconic marine invertebrate, *C. sapidus*, directly consumes vegetative material of all SAV species tested, even if only as relatively small components of their overall diet. The continued persistence of non-native SAV despite *C. sapidus* consumption, and that of other herbivores present, also indicates that herbivory in the system is sufficient to prevent the recovery of some, but not all, SAV species. The ability of some SAV species but not others to overcome grazing may stem from the growth rate and reproductive traits of a given SAV species.

Chapter Three

Chapter Three explores whether seedling establishment and recruitment is important in the maintenance of *Zostera marina* meadows in Chesapeake Bay and whether interactions between seedlings and adult shoots impact seedling survival within *Z. marina* meadows. Populations of *Z. marina* in Chesapeake Bay annually produce large numbers of seed, and many of these seeds remain in the vicinity of their parent plant (Silberhorn et al. 1983, Harwell & Orth 2002, Manley et al. 2015). Because *Z. marina* populations exhibit large fluctuations in growth and biomass seasonally and spatially in Chesapeake Bay (Orth & Moore 1986), seeds germinating amongst adult shoots could experience variable competition for limiting resources with surrounding shoots. If seedlings survive within existing *Z. marina* meadows, sexual reproduction would play not only an important role in meadow recovery from catastrophic diebacks (Jarvis & Moore 2010), but perhaps the annual maintenance of meadows.

To gauge the extent to which sexual reproduction occurs in *Z. marina* meadows and the influence of adult vegetation on seedling survival, surveys were first used to estimate the number of seedlings establishing among adult shoots within four *Z. marina* meadows of the lower York River, Chesapeake Bay over three years. Simultaneously, a seed addition experiment was used to test if the size of the seed bank influenced the number of seedlings establishing. An additional survey evaluated if the percent bottom cover, shoot density, and shoot height of surrounding adult vegetation impacted seedling survival. A separate experiment then directly compared the survival of seedlings growing among neighboring *Z. marina* shoots to the survival of seedlings growing without

neighboring shoots. These studies do not explicitly test for density-dependent effects of adult shoots and seedlings on one another. Instead these studies evaluate competition as the outcome of all interactions between adult shoots and seedlings that may compromise seedling survival and potentially impact seedling recruitment patterns. Last, experimental *Z. marina* plots with and without sexual reproduction were established in bare sediment just upstream from an existing meadow. The percent bottom cover of these plots was then tracked to determine if sexual reproduction was important in the maintenance of *Z. marina* meadows in Chesapeake Bay. Results from this work showed seedlings consistently establishing in *Z. marina* meadows in Chesapeake Bay and that seed supply could influence the rate of seedling establishment. Surveys comparing seedling survival across meadows with different adult shoot characteristics and experimental manipulations of neighboring shoots suggest adult shoots lower the survival of seedlings growing in their proximity. Sexual reproduction may be an irregular, but important, source of genetic diversity and propagules for existing *Z. marina* populations in Chesapeake Bay. Disturbances to adult vegetation, prior to seedling establishment, may lower interactions between seedlings and surrounding vegetation, and increase both seedling survival and the overall importance of seedling recruitment to *Z. marina* populations.

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CHAPTER ONE

A sediment bioturbator bottleneck to seedling recruitment for the seagrass *Posidonia australis*[*]

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Abstract

Bioturbating animals have the potential to influence the distribution and survival of seagrass seeds and seedlings within unvegetated substrates. Such disturbances could act as demographic bottlenecks, or restrictions, to seedling recruitment and impede population recovery in degraded systems. This study evaluated the influence of sediment bioturbators on seed settlement and seedling establishment for the seagrass *Posidonia australis* in temperate and subtropical areas of Western Australia, Cockburn Sound and Shark Bay. Initial benthic surveys recorded the density of sediment bioturbators as well as *P. australis* seeds and seedlings. The abundant bioturbators observed were: the sand dollar (*Peronella leseuri*) and the sea star (*Archaster angulatus*) in Cockburn Sound, and the heart urchin (*Breynia desori*) in Shark Bay. Surveys demonstrated an overlap in habitat use among bioturbators, seeds, and seedlings and suggested potential negative seed-fauna interactions in areas where bioturbators were present and abundant. To test this relationship, field manipulative experiments measured the rate at which these bioturbators dislodged and moved *P. australis* seeds, both unburied and buried, and one-year-old seedlings. Although all bioturbators dislodged and moved seeds on the sediment surface, only sand dollars and heart urchins dislodged and moved seeds (on average ~ 4cm) buried within the sediment. Where high densities of bioturbators, such as sand dollars and heart urchins, exist, considerable impact on *P. australis* seeds would be expected. No animals dislodged one-year-old seedlings. Sediment bioturbators act as an important but often overlooked, bottleneck to seedling recruitment and recovery of degraded seagrass communities.

KEY WORDS: bioturbators, seeds, seedling recruitment, restoration, seagrass, *Posidonia australis*

Introduction

Seeds and seedlings are critical stages in the life history of seagrasses, particularly when the stability, persistence, and recovery of populations are dependent on seedling recruitment (Kendrick et al. 2017). Because early demographic stages are associated with high mortality rates, they may be considered demographic "bottlenecks" which can limit recruitment within populations (Bond 2008, James et al. 2011, Statton et al. 2017). Seedling recruitment may be particularly rare in degraded systems even after the conditions prompting the degradation have been remedied (Suding et al. 2004). Once vegetation has been lost, changes in micro-climate, nutrient availability, hydrology, hydrodynamics, predator-prey and/or plant-herbivore interactions can limit seed or seedling survival (Janzen 1971, Harper 1977, Eriksson & Ehrlen 1992, Fishman & Orth 1996; Holbrook et al. 2000, Wenny 2000, Orth et al. 2002, 2006b, 2007; Alagna et al. 2013, Darnell & Dunton 2015, Manley et al. 2015). In the marine environment, benthic detritivores and scavengers that are obligate bioturbators of bare substrate are an additional threat to newly settled seagrass seeds and seedlings (Dumbauld & Wyllie-Echeverria 2003, Valdemarsen et al. 2011, Statton et al. 2012). Seagrass meadows have become increasingly degraded from human disturbances (Orth et al. 2006c, Waycott et al. 2009), resulting in unvegetated substrates where bioturbating fauna can potentially play a major role in influencing seed settlement and seedling recruitment as environmental conditions improve. Therefore, a clear understanding of how these seed- and seedling-fauna interactions influence early recruitment success is a critical step in providing appropriate management approaches to improve recovery and regeneration of lost seagrass habitat.

Bioturbation, the biological reworking of sediments, can pose a significant constraint on seedling recruitment and thus the natural regeneration or restoration of seagrass communities (Suchanek 1983, Phillipart 1994, Meysman et al. 2006, Statton et al. 2012). Although bioturbators may not directly consume a seed or seedling, disturbance of the sediment may uproot, bury, or damage them. Such disturbances, however, may not always be harmful or could result in differing outcomes depending on the seagrass life-stage. For instance, polychaete and crustacean deposit feeders have been shown to both positively influence seedling recruitment through shallow burial of seeds (Luckenbach & Orth 1999, Blackburn & Orth 2013) but negatively influence seedling establishment through deep burial or light limitation (Dumbauld & Wyllie-Echeverria 2003, Valdemarsen et al. 2011). Several studies have suggested polychaete bioturbators may have a profound impact on seagrass meadow distribution and health for *Zostera spp.* (Davis & Short 1997, Hughes et al. 2000, Delefosse & Kristensen 2012), but relatively few studies have explored the impact of other bioturbator assemblages (for example crustaceans, Dumbauld & Wyllie-Echeverria 2003, Molenaar & Meinesz 1995) on key demographic shifts of other seagrass species with different growth habits than *Zostera* spp.

Bioturbators are a diverse and active group of organisms with an equally diverse range of behaviors that disturb the sediment. Estimates of bioturbation on a global scale suggest bioturbating fauna may rework enough sediment in a year to bury metropolitan London in 13km of sediment (Teal et al. 2008). Different species will, however, rework or scour sediment differently in search of food or burrow into the sediment for refuge (Kristensen et al. 2012). Polychaetes in coastal marine environments irrigate or consume

sediments and have been reported to rework 91-114 $cm^3\ m^{-2}\ day^{-1}$ $individual^{-1}$ (Valdemarsen et al. 2010). Spatangoida urchins push themselves through the upper 5 cm of the sediment as they feed and have been reported to rework up to 500 $cm^3\ m^{-2}\ day^{-1}$ $individual^{-1}$ (Lohrer et al. 2005). A diverse global bioturbator community could thus have a substantial, but variable, impact on aquatic vegetation communities also inhabiting sediments.

For the seagrass *Posidonia australis*, a dominant seagrass species found in the temperate coastal waters of southern Australia, seeds are important for the dispersal of seedlings to remote and/or uncolonized habitat but are also integral to the population dynamics and genetic composition of existing populations (Kendrick et al. 2012, 2017, Sinclair et al. 2014). *P. australis* is a slow growing and long-lived, "persistent," foundation habitat that flowers annually to produce positively buoyant fruit capable of dispersing large distances before releasing a large (1.5 – 2.0 cm) and directly developing seed (Montoya et al. 2012, Kilminster et al. 2015, Statton et al. 2017). For the purposes of this paper, the term "seed" will refer to this recently released propagule without a root-rhizome network anchoring it into the sediment. A "seedling" will refer to a propagule with a root-rhizome network within the sediment. "Seedling establishment" will refer to the process by which a seed transitions into a seedling, and "seedling recruitment" will describe the entire process by which a seed ultimately enters the adult, reproductive population. Because *P. australis* seeds are growing from the moment of release, seeds have a narrow window of time to grow root anchors. Statton et al. (2017) suggested the majority of seed mortality in sheltered and moderately exposed locations occurs in less than one month and hypothesized this mortality resulted from abundant echinoderm

populations at these locations. Based on the shallow tracks and pits they observed echinoderm bioturbators generate in the sediment at depths suitable for seed settlement, they suggested these animals dragged or pushed seeds out of the sediment and away from restoration plots. Unlike previously studied bioturbator species that compromised seed and seedling survival through burial below a previously determined critical sediment depth, these bioturbators could potentially pull seeds and seedlings out of the sediment. For *P. australis*, uprooting seeds has profound implications on seed survival as shoot development can make a seed positively buoyant in as little as two weeks (*personal observation*, Figure S1). Bioturbator foraging which pushes seeds out of the sediment during critical early life stages could thus have strong effect on *P. australis* seed survival as seeds pushed out from the sediment or prevented from anchoring themselves to the sediment with roots will float away from suitable habitat. In addition, because *P. australis* exhibits a "phalanx" growth style of densely packed shoots (Lovett-Doust 1981) seedling recruitment is assumed most likely to occur in unvegetated areas between or away from adults that potentially compete with seeds for limiting resources. Similarly, the short-term survival of *P. australis* seeds in bare sand was 3-5 fold higher than within *P. australis* meadows abundant with seed predators (Orth et al. 2002, 2006d, 2007). Thus, seed settlement and seedling recruitment may be limited in space and time to bare areas that are also suitable habitat for sediment bioturbators in search of food or refuge.

In this study we evaluated the interaction between sediment bioturbators, recently settled seeds, and one-year-old seedlings of the seagrass *Posidonia australis* using both laboratory and field experiments. The objectives were: 1. To survey the bioturbator species present in both natural and restoration settings, their densities, and their spatial

overlap with *P. australis* seeds and seedlings in temperate western Australia; 2. To quantify the rate and categorize the type of movement for each sediment bioturbator; 3. To determine if bioturbators are capable of dislodging and moving seeds that are settled on the surface or pushing seeds buried in the sediment out of the sediment; 4. To determine if bioturbators are capable of dislodging one year old seedlings. By evaluating the interaction between bioturbators and recently settled seeds and one-year-old seedlings in this way, we expected to find a suite of bioturbators cause a significant bottleneck to seedling recruitment and thus also to the recovery of degraded *P. australis* communities.

Methods

Study Sites

Our research was conducted within two embayments (Figure 1) with several sites in each; Cockburn Sound (Cockburn Sound East, Owen Anchorage North, Owen Anchorage Central, Owen Anchorage South, and Carnac Island) and Shark Bay (Useless Loop and Guichenault Point), Western Australia. Cockburn Sound (−32.135356, 115.731646) and the surrounding area, is a temperate semi-enclosed embayment near Perth and mid-range in the distribution of *Posidonia australis* along the coast of Western Australia. Shark Bay (−26.113597, 113.411681), is a shallow, subtropical embayment and represents a population of *P. australis* near its northernmost limit on the west coast of Australia. Survey sites and experimental trials focused on bare sandy areas adjacent to existing meadows of *P. australis* and at 2-4 m depth. These sites are part of ongoing seagrass recovery and restoration programs.

Study Design

To achieve the objectives outlined above, four separate but linked investigations were implemented as follows: 1. Surveys were conducted to establish the relative abundance of bioturbators and their overlap in distribution with seeds and seedlings of *P. australis;* 2. Observations of the movement of the three most abundant bioturbators, the sand dollar *Peronella lesueuri* (Agassiz 1841) and sea star *Archaster angulatus* (Müller & Troschel 1840) in Cockburn Sound, and the heart urchin, *Breynia desori* (Gray 1851), in Shark Bay, were measured in laboratory and field environments; 3. *In situ* experiments were then conducted to estimate the disturbance and disruption of bioturbator movement on seeds of *P. australis*; 4. *In situ* experiments were conducted to determine if one-year-old seedlings with more developed root structures were disturbed by the movement of sand dollars and sea stars.

Survey of Fauna and Recently Settled Seedlings

To measure the overlap and abundance of sediment bioturbators and *P. australis* seeds and seedlings, we surveyed five bare sandy areas representing areas of potential seedling recruitment at two locations, Cockburn Sound and Shark Bay, for suspected bioturbators and *P. australis* seeds and seedlings in autumn 2016. Suspected bioturbators were animals impacting the upper 2 cm of sediment through their movement. Recently settled *P. australis* seeds are ~2 cm long and may already maintain an ~1 cm tall shoot, making them easily identifiable on the sediment surface or shallowly buried (Statton et al. 2017). Individual plants with 1-2 shoots were identified as one-year-old seedlings. Four sites in Cockburn Sound previously identified in a large scale restoration program were surveyed; Owen Anchorage North, Central, and South, and Cockburn Sound east, and one unvegetated reference site on the shoreward, eastern side of Carnac Island. In Shark

Bay, three sites were selected within Useless Loop, which were also part of ongoing restoration trials, and two bare sandy reference sites at Guichenault Point. Reference sites at both locations were adjacent to flowering *P. australis* meadows and were included in the survey because each site potentially experiences less benthic bioturbating fauna than the restoration sites. At each site, five 10 × 1 m transects were randomly assigned within bare sediments adjacent to *P. australis* meadows. Divers swam along these transects with a one-meter bar and recorded all animals on the surface and just below the sediment surface by sight and gently patting the sediment surface within one meter of the transect. For seed and seedling counts, we conducted the survey in December after *P. australis* seed release to record the number of recently settled seeds and ~1 yr old seedlings (Statton et al. 2013).

Animal Movement

Laboratory Experiments

To estimate the relative intensity with which the most disruptive, mobile, and abundant sediment bioturbators disturb the sediment surface, we conducted movement experiments within controlled, laboratory conditions which allow the collection of more detailed information on bioturbator movement through time than would be feasible over the duration of *in situ* experiments on SCUBA. Sand dollars were selected for these detailed measurements of movement as they were known to move while partially buried within the upper ~1 cm of the sediment and have been recorded at densities as high as 6 individuals m^{-2} in Cockburn Sound (Forehead & Thompson 2010). Seven sand dollars were collected and placed within two 1800 L recirculating aquaculture tanks containing two 0.5 m^2 tubs filled with sediment until 20 cm below the water line. After sand dollars

were acclimated to tank conditions for 24 hours, the sand dollars were positioned at the end of each tub and small plastic position markers were placed behind the sand dollars every two hours for 6 hours and again the following morning. The linear distance (cm) between position markers was then measured. The sand dollars were tracked over three days and position markers repositioned each day.

Because measuring the linear distance between plastic position markers placed behind sand dollars at regular intervals did not account for the nonlinear movements of sand dollars, three additional sand dollars were collected from Cockburn Sound and placed within one of three 0.5 m^2 tubs (described above) and acclimated for 24 hours before estimating their nonlinear movement through time. After acclimating, each sand dollar was moved into an identical tub in which the sediment was covered with an additional 1 cm of fine white silica sediment. The underlying sediment was red-orange in color for maximum contrast when bioturbation was observed. Each tub was divided in half (2×0.25 m^2 areas) and one sand dollar was placed into one half of each tub while the adjacent half received no sand dollar and served as a control (i.e. no sediment disturbance from sand dollars). A position marker was placed behind the sand dollar as it was introduced into the tank. Every six hours a photo of all three tubs was taken and the percent of disturbed fine sediment in each tub over which the sand dollars had moved, as well as the linear distance the sand dollars had traveled, was recorded for 24 hours.

Field Experiments

The most abundant bioturbators recorded in the transect surveys, sand dollars (*P. lesueuri*) and sea stars (*A. angulatus*) in Cockburn Sound, and heart urchins (*B. desori*) in Shark Bay, were selected for detailed estimates of their movement rates. *In situ* estimates

of the rate at which sand dollars, sea stars, and heart urchins move were recorded during bioturbator experiments on SCUBA (*see* *Bioturbator Experiments - Recently Settled Seeds*). These experiments used plastic dividers (7.5 cm H, 50 cm L) inserted into the sediment to guide individuals of each bioturbator species from a fixed point, one end of the plastic dividers, into three seeds placed across each lane 20 cm in front of this point and within the path generated by the dividers for each bioturbator (Figure 3). An experiment was concluded when an animal had either moved beyond all of the seeds in its path or moved 50 cm and thus beyond the plastic dividers guiding its movement. The rate at which a given animal moved during the experiment was calculated by measuring the duration over which each experiment occurred and the total distance each animal moved during the experiment, from initial placement in the experiment at a fixed location to the animal's position at the end of the experiment. Because heart urchins move beneath the surface and more slowly than sand dollars and sea stars, *in situ* observations of heart urchin movement within a day were not feasible. As a result, heart urchins were left for 24hrs and the distance the animals moved over this time period was recorded.

To estimate how frequently sand dollar and sea star populations may disturb *P. australis* seeds, the density, size, and movement of each bioturbator species was multiplied by the mean density of seeds at a survey location with high abundances of both species, Owen Anchorage Central (OAC). The mean rate at which individuals moved in experiments was scaled to a daily rate and multiplied by the mean width for each species to calculate a mean area the individuals of each species would disturb in a day. This calculated disturbance rate was then multiplied by the density of each species at OAC to calculate the area the sand dollar or sea star population at OAC would disturb in

a given day. This disturbed area was then multiplied by the mean number of *P. australis* seeds counted m^{-2} at OAC to estimate how many natural seeds each species would disturb at this location in a given day. In order to estimate how many seeds these animal populations would disturb daily in theoretical 50 m^2 restoration plots at OAC in Cockburn Sound or at Useless Loop (UL1, Figure 4d) in Shark Bay, the area each population of bioturbator would disturb daily was multiplied by 100 seeds m^{-2}, the seeding density used in Statton et al. (2017) as a proposed seeding density.

Field Bioturbator Experiments - Recently Settled Seeds

To evaluate the impact of sediment bioturbator movement on *P. australis* seeds recently settled on the sediment surface and also buried within the sediment, *in situ* field experiments were conducted at both Cockburn Sound and Shark Bay. We tested the influence of the sand dollar, *P. lesueuri*, and the sea star *A. angulatus*, in Cockburn Sound and the heart urchin *B. desori* in Shark Bay. Raceways or lanes were constructed in bare sand using two, 7.5 cm H, 50 cm L plastic dividers inserted into the sediment 20 cm apart. Individual bioturbators were placed at the start of a raceway and three recently settled seeds were placed across each row 20 cm from the base of the lane (Figure 2 & 3). As each animal moved along the lane, the impact of the animal's movement on the seed's state was categorized as either disturbed (uprooted or buried as a consequence of the bioturbator's movement) or undisturbed (unaffected or unimpacted by the bioturbator's movement). Control lanes without bioturbators were also established alongside lanes with bioturbators to evaluate if currents or alternative processes may also have moved seeds. If after 3 minutes a sand dollar or sea star had not moved from the start of the lane, the animal was deemed unresponsive and replaced with an individual of the same species

that was mobile. An experiment was concluded when an animal had either moved beyond all of the seeds or moved > 50 cm and thus beyond the plastic dividers guiding its movement. Because heart urchins move beneath the sediment surface and more slowly than sand dollars and sea stars, heart urchins were left within the experimental lane for 24 hours before evaluation. The distance a seed was moved, either by animal or other processes, was measured in 3 cm increments up to 15 cm from the seeds starting position. Seven sea stars, eight sand dollars, and nine heart urchins were guided into seeds placed on the sediment surface. Nine animals of each species were guided into seeds buried 1 cm into the sediment. After all animals had finished interacting with the seeds, the length, width, and height of each animal was recorded. Additionally, to determine if animals interacted with seeds when animals were not handled or guided into seeds by lanes, three seeds were placed 5 cm in front of unhandled and moving sand dollars and sea stars and then recorded the seed state after the interaction.

Field Bioturbator Experiments - One-year-old Seedlings

To test if bioturbators were able to disturb one-year-old seedlings, plastic divider raceways (as described in *Bioturbator Experiments - Recently Settled Seeds*) were also constructed around one-year-old seedlings in Owen Anchorage. Nine sand dollars and nine sea stars were introduced at the base of each lane and guided into a single one-year-old seedling centered 20 cm from the start of each lane. Whether the seedling was disturbed or undisturbed was then recorded, and, if disturbed, the distance the animal moved the seedling was measured. This experiment was not conducted with heart urchins in Shark Bay as one-year-old seedlings were not present within the experimental area.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine if significant differences in the densities of sand dollars, sea stars, *P. australis* seeds, and *P. australis* seedlings occurred between surveyed locations in Cockburn Sound. Spearman's rank correlation coefficients were then used to test if any statistically significant relationships existed between the mean number of bioturbators and either the mean number of *P. australis* seeds or seedlings observed at survey locations in Cockburn Sound. Separate correlations tested if the mean number of seeds counted at a location was related to the mean number of sand dollars or sea stars also observed at that location. Additional correlations tested whether the mean number of one-year-old seedlings counted at a location was related to the mean number of sand dollars or sea stars at that location. These correlations were not intended to determine causality between animal densities and seed or seedling densities, but rather to determine if any statistically significant relationship exists between *in situ* sediment bioturbator populations and the presence of early seagrass life stages. Spearman's rank correlation was used as a non-parametric, rank-based alternative to Pearson's Product moment correlation as the surveyed animal and plant densities were not normally distributed. Correlation analysis was not conducted with heart urchins in Shark Bay, as heart urchins were not recorded in the vicinity of *P. australis* seeds outside restoration areas.

To determine if sand dollars scouring the fine sediment in experimental tubs disturbed more of the upper 1 cm of the sediment than in control areas, differences in the percentage of sand disturbed between sand dollar and control areas were analyzed with a repeated measures ANOVA.

Because seeds or seedlings placed in experimental lanes were categorized as disturbed (uprooted or buried) or undisturbed (left in state), odds ratios were used to compare the likelihood of a seed or seedling being disturbed in lanes with bioturbators relative to the likelihood of a seed or seedling being disturbed in lanes without a bioturbator for each species of bioturbator evaluated. An odds ratio of 1 would indicate seeds or seedlings were as likely to be categorized as disturbed in lanes with bioturbators as in lanes without bioturbators. Fisher's exact tests were then used to estimate if observed disturbance was significantly different than expected (i.e. the disturbance would be the same) in animal and control lanes. To test if seeds or seedlings in experimental lanes with animals moved more than seeds or seedlings within control lanes, Welch's t-tests were used to determine if significant differences in the mean distance seeds or seedlings moved existed between lanes with or without animals. Welch's test was used as the variance between animal and control treatments was uneven.

A type I error rate of 0.05 was established for all statistical tests. Spearman's rank correlation coefficients were conducted using the *cor.test* function from the *stats* R core package (R Development Core Team 2015). Odds ratios and fisher's exact tests were calculated with the *oddsratio.wald* function within the *epitools* package (Aragon 2012). All statistics were performed in R statistical analysis software (R Development Core Team 2015).

Results

Survey of Fauna and Recently Settled Seedlings

Surveys of bioturbating animals recorded the highest densities of the sea star (*Archaster angulatus*) and the sand dollar (*Peronella lesueri*) in Cockburn Sound East and the lowest densities at Carnac Island. Blue swimmer crabs (*Portunus armatus*) were also observed at Owen Anchorage South but were not abundant (n=3) or observed at other locations. At Useless Loop in Shark Bay the heart urchin (*Breynia desori*) occurred at the highest densities. Hermit crabs (*Pagurus spp.*), decorator urchins (*Tripneustes gratilla*), and the blue swimmer crab (*Portunus armatus*) were also recorded at Useless Loop. Only one hermit crab (*Pagurus sp.*) and several molts of the blue swimmer crab were observed at Guichenault Point.

In Cockburn Sound, the most abundant bioturbating species disturbing the upper centimeters of sediment were the sand dollar *Peronella lesueri* and the sea star *Archaster angulatus*. Their densities varied significantly, however, between sites (Figure 4a; $F_{4,20}$ = 32.9, $p < 0.001$; $F_{4,20}$ = 14.5, $p < 0.001$; Table S1; Table S2). Sand dollars were present in Cockburn Sound East (8.6 ± 1.6 sand dollars per transect), Owen Anchorage Central (7.8 ± 1.3 sand dollars per transect), and Owen Anchorage North (1.6 ± 0.6 sand dollars per transect). Sea stars were also present with sand dollars at Owen Anchorage Central (11 ± 1.3 sea stars per transect) and Cockburn Sound East (2.0 ± 1.6 sea stars per transect), but were the dominant bioturbator present at Owen Anchorage South (8.6 ± 0.2 sea stars per transect).

Recently settled seeds were found at significantly different densities (Figure 4b; $F_{4,20}$ = 94.9, $p < 0.001$; Table S3) at sites surveyed around Cockburn Sound. Carnac Island and Owen Anchorage South recorded the highest densities of seeds per transect (94 ± 17 and 71 ± 8.4, respectively); while Owen Anchorage North (11 ± 2.6) and Central

(4.6 ± 1.8) and Cockburn Sound East (8.0 ± 2.1) recorded far lower densities of seeds per transect. One-year-old seedlings also varied significantly in density across sites ($F_{4,18}$ = 8.73, $p < 0.001$; Table S4). Owen Anchorage Central had the highest number of seedlings per transect (9.4 ± 2.0), while Cockburn Sound East (3.7 ± 2.7), Owen Anchorage South (3.0 ± 0.95), Carnac Island (2.2 ± 0.58), and Owen Anchorage North (1.4 ± 0.75) exhibited lower densities of seedlings per transect.

Posidonia australis seeds and seedlings were found together with bioturbating fauna within Cockburn Sound and Owen Anchorage but not at Carnac Island (Figure 4b). A significant negative correlation was detected between the density of sand dollars at a survey site and the density of seeds at that site (rho = −1, S = 40, p = 0.02, Figure 4c; Table 1). This correlation suggests the potential for a negative relationship between sand dollar density and seed settlement at surveyed locations. The abundance of recently settled seeds was lowest at sites with high sand dollar densities in Cockburn Sound East and Owen Anchorage Central whereas the highest abundance of recently settled seeds were at sites with low densities of sand dollars (Carnac Island and Owen Anchorage South). No significant correlation, however, was detected between the density of sand dollars and the number of one-year-old seedlings at a surveyed location or between the density of sea stars and either the number of seeds or the number of one-year-old seedlings at a surveyed location (Table 1).

At Useless Loop, in Shark Bay, the most abundant bioturbator was the heart urchin *Breynia desori.* This species is known to rework the upper 2-4 cm of sediment. During surveys heart urchins were found at low densities (2 ± 0.4 urchins per transect or 0.18 urchins per m^2; Figure 4d) at all sites in Useless Loop but have been found at much

higher densities (2-3 m^{-2}, Statton pers obs). Heart urchins were absent from Guichenault Point. Only two recently settled *P. australis* seeds were observed at Guichenault Point. No *P. australis* seeds or seedlings were found at Useless Loop.

Animal Movement

Laboratory Experiments

The seven sand dollars removed from Cockburn Sound and tracked within tanks moved 2.4 ± 4.2 cm hr^{-1} (range: 0 – 26 cm hr^{-1}). These sand dollars in tanks moved substantially less than sand dollars in the field (~13 ± 4.8 cm hr^{-1}, n = 9).

Over 24 hours, the three sand dollars placed in tanks with fine sediment covering the surface disturbed ~0.15 m^2 (55% ±10, n=3, Figure 4) of the sediment surface and disturbed significantly more of the upper 1 cm of fine sediment relative to control tanks without sand dollars ($F_{1,27}$ = 16.6, p < 0.001).

Field Experiments

Rates of animal movement and dimensions of animals calculated from field experiments indicate all three species interacted with the upper 1-4 cm of sediment over the course of the experiment. Sand dollars partially buried themselves within the sediment (~1cm) and were 2.2 (± 0.2) cm tall, 13 (± 0.3) cm wide, and 15 (± 0.2) cm long and moved on average 13 (± 4.8) cm hr^{-1} with a maximum recorded rate of 82 cm h^{-1}. Sea stars moved primarily over the surface of the sediment and were 1.1 (± 0.02) cm tall, 17 (± 0.2) cm wide, and 18 (± 0.3) cm long and moved approximately 33 (± 8) cm $hr^{-1}$with a maximum recorded rate of 90 cm hr^{-1}. Heart urchins were 3.7 (± 0.8) cm tall, 6.4 (± 0.1) cm wide, and 8.6 (± 0.1) cm long. After handling the urchins to introduce them at the base of a lane, all urchins immediately buried themselves ~4cm within the

sediment. Urchins then moved within the sediment for an average distance of 29.3 cm (± 0.3, range: 23 – 35 cm day^{-1}, ~1.2 cm hr^{-1}) within a day.

Measurements of sand dollar movement in laboratory conditions and *in situ*, suggest individual sand dollars could disturb ~0.15 - 0.4 m^2 day^{-1} respectively, and their populations could disturb between 6-16 m^2 of the survey area and 3-8 seeds, or 13-35% of all recorded seeds, daily at the densities recorded at OAC. Individual sea stars moving across the sediment could disturb ~1.35 m^2 day^{-1} and the sea star population at OAC could disturb ~75 m^2 and ~38 seeds, 100% of recorded seeds, per day. Last, heart urchins observed in Shark Bay could disturb ~0.4 m^2 per day at their highest densities recorded in this study, however, at higher observed densities of 2-4 urchins m^{-2} (J. Statton, *personal communication*), urchins could disturb ~3 m^2 per day. If 50 m^2 restoration plots at OAC in Cockburn Sound or UL1 in Shark Bay were broadcast with 100 seeds m^{-2}, sand dollars could disturb 600 – 1600 seeds daily (12-32% of the seeds in the 50m^2 plot), sea stars could disturb 7500 seeds daily (100% of the seeds in the 50 m^2 plot), and heart urchins could disturb 40-300 seeds daily (0.8 – 6% of the seeds in the 50 m^2 plot).

Field Bioturbator Experiments – Recently Settled Seed

The three species of bioturbators exhibited different movement characteristics, affecting how they disturbed seeds on the surface versus seeds buried within the sediment. All three species of sediment bioturbators examined were capable of dislodging recently settled seeds in both Cockburn Sound and Shark Bay. The odds of a seed being disturbed were significantly higher in lanes with fauna present; sand dollars (odds ratio: 50.4, 95% CI: 8.8-290, $p < 0.001$), sea stars (odds ratio: 170, 95% CI: 14-2100, $p < 0.001$), and heart urchins (odds ratio: 9.2, 95% CI: 2.3-38, $p = 0.001$), than in their

respective control lanes where animals were absent. Burial of seeds within lanes provided some refuge from disturbance, but the odds of buried seeds being disturbed in lanes with sand dollars (odds ratio: 26, 95% CI: 3.1-220, $p < 0.001$), sea stars (odds ratio: 8.9, 95% CI: 1.0-78, $p = 0.05$), and heart urchins (odds ratio: 3.5, 95% CI: 4.7-330, $p < 0.001$) was still significantly higher than the odds of buried seeds being disturbed within control lanes.

The mean distance a seed moved on the surface was also significantly higher in lanes with sand dollars ($t_6 = 9.4$, $P < 0.001$), sea stars ($t_3 = 5.2$, $P = 0.01$), and heart urchins ($t_{12} = 3.0$, $P = 0.009$) than in control lanes (Figure 5 & S2; Table 2). Burial of seeds again provided some refuge for seeds as the mean distance seeds moved was significantly higher in lanes with sand dollars ($t_8 = 3.1$, $P = 0.01$) and heart urchins ($t_8 = 6.0$, $P < 0.001$) relative to control lanes (Table 2). Lanes containing sea stars did not show significantly higher movement of seeds than control lanes ($p = 0.10$). Sand dollar and sea star disturbance exclusively pushed seeds on the sediment surface or uprooted seeds initially buried 1cm within the sediment. Interestingly, of the seeds initially buried, heart urchins dislodged 56% of the seeds (n=9), and pushed 44% of the seeds beneath the sediment surface (n=7). Three seeds initially placed on the sediment surface were also found buried in urchin lanes.

The movement and disturbance of seeds placed within the path of animals outside experimental constructs was observed and demonstrated that animals disturbed seeds within their path equivalent to animals used within the experimental trials (Figure S3).

Field Bioturbator Experiments - One-year-old Seedlings

Both sand dollars and sea stars were unable to dislodge or move one-year-old seedlings from the center of the lane, instead they either moved around or stopped moving when they encountered a seedling. After each trial with an animal, one-year old seedlings were excavated, and well established roots anchoring the seedling in sediment were observed.

Discussion

We have demonstrated using field observations and field and laboratory experiments that sediment bioturbator communities in temperate and subtropical environments of Western Australia can dislodge recently settled seeds and act as a bottleneck to seedling establishment for a persistent and slow growing seagrass species, *Posidonia australis*. The risk of bioturbators affecting one-year-old seedlings, however, was minimal. These results suggest that if seeds can recruit and survive past the early stages of development, they may persist and contribute to meadow development, but only if other drivers of recruitment failure, whether they be biotic or abiotic, are absent or have been mitigated (Statton et al. 2017).

Co-occurrence of Sediment Bioturbators and Seeds

Bioturbators are common in soft sediments around the world (Kristensen et al. 2012) and have been well documented in, and adjacent to, seagrass meadows. The impact of their presence on seed and seedling recruitment will be a function of the abundance, location and behavior of each bioturbator species (Valdermarssen et al. 2011, Delefosse & Kristensen 2012, Blackburn & Orth 2013, Statton et al. 2017). Our *in situ* surveys and experimental observations found that bioturbating species in unvegetated areas adjacent

to seagrass meadows in Western Australia overlapped in space and time with recently released *P. australis* seeds and showed the potential for a negative relationship between the abundance of certain bioturbators and seed presence.

These observations and experiments support previous research suggesting high densities of bioturbators will disturb recently settled seeds recruiting into degraded or unvegetated sediment habitats, and demonstrate the mechanism of disturbance can be diverse. The feeding and defecation of burrowing sediment detritivores, such as the polychaetes *Nereis diversicolor* and *Arenicola marina*, have buried seeds and seedlings of *Zostera noltii* and *Zostera marina* below their critical depth for survival. This burial has been implicated as a major process that has slowed the recovery of both species (Phillipart 1994, Hughes et al. 2000, Valdemarsen et al. 2011). A similar burrowing worm on the south coast of Australia has damaged *Posidonia australis* transplants in bare sediment and would likely bury any seeds or seedlings in their vicinity (Bastyan & Cambridge 2008). Similarly, thalassinid shrimp burrowing in search of food and shelter have prevented seedling establishment of *Zostera japonica* in bare sediment recently released from aquaculture production in the Pacific Northwest of the United States (Dumbauld & Wyllie-Echeverria 2003). For these bioturbators, the relative impact of the bioturbator community on seedling recruitment stems from the density of bioturbators feeding or defecating in the vicinity of seeds as mortality stems from seed or seedling smothering adjacent to animal burrows. This study demonstrates a new group of echinoderm bioturbator species which act like bulldozers scrapping either at the surface or subsurface sediments and uprooting recently settled seeds in their path. For this community, the impact of the bioturbators stems from both the density and mobility of

the species present and pushing seeds around or out of the sediment where seeds are exposed to seed predators and further disturbance (Orth et al. 2002, 2006d, 2007). Because these echinoderm bioturbators are mobile, they may encounter multiple seeds or even the same seed on multiple occasions in a given day while foraging. These findings suggest the mechanism of seed disturbance will vary between bioturbator communities, largely based on the mobility and behavior of the species present.

Seed dormancy and germination traits will also be critical to understanding seedling establishment patterns in the presence of bioturbators. *P. australis* produces viviparous seeds with no dormancy (Orth et al. 2000) and thus only a short window for seed settlement and establishment (Statton et al. 2017). Because mobile, echinoderm bioturbators are not only capable of pushing seeds settled on the sediment surface but also of pushing seeds out of the sediment and back to the sediment surface, their activity at high densities may keep seeds on the surface without roots anchoring them in the sediment during periods of rapid leaf growth. This persistent disturbance of large and germinated *P. australis* seeds on the sediment surface may ultimately prevent incorporation in the sediment and the development of sufficient anchors to prevent growing seeds from floating away to unsuitable habitats. Our results demonstrating the consequences of bioturbator disturbance, in combination with the impacts of insufficient seed anchoring, may thus explain the low survival of seeded plots in sheltered locations with abundant bioturbator communities (Statton et al. 2017). Alternatively, seagrass species with seeds that have some form of dormancy, e.g. *Zostera* spp. (Orth et al. 2000), may initially survive secondary dispersal events as a result of bioturbation, but ultimately may suffer similar fates to species with no dormancy, depending on their physical

location within the sediment when they do germinate. The relative impact of incidental bioturbator disturbance will thus likely be larger in the short term for seeds without a dormancy period than for dormant seeds which may survive several disturbance events and for whom survival will be dictated over a longer period of time. Seeds with dormancy may, however, encounter additional predation pressure over the length of their dormancy period that also reduces the number of viable seeds in the seed bank and leads to lower seedling establishment rates.

But these studied bioturbators are a small subset of the global and diverse bioturbating community that are capable of disturbing seeds and/or seedlings. Larger bioturbators may also disturb the sediment when they forage for infauna. The green crab (*Carcinus maenas*) disrupted and uprooted *Z. marina* transplants in New England, USA (Davis et al. 1998). The portunid crab *Callinectes sapidus* is a known bioturbator of *Zostera marina* meadows in the Atlantic (Wilson et al. 1990) and a relative, *Portunus armatus*, is a common bioturbator in Western Australia and was observed disturbing bare sediment during this study (Figure S4). Likewise, elasmobranches are known to dig into both bare and vegetated patches in search of food and generate large escarpments in the sediment (Orth 1975, Townsend & Fonseca 1998). Previous studies indicate these elasmobranch bioturbators may (Orth 1975, Backman 1984, Fonseca et al. 1994, Inglis 2000) or may not (Valentine et al. 1994) be able to disturb adult clones of seagrass species, but these studies have not incorporated seeds and seedlings which would be more susceptible to damage and loss from elasmobranch foraging. The widespread and diverse nature of bioturbators suggests these communities may play a more substantial role in seagrass seedling recruitment than currently acknowledged.

Bioturbator Movement and Burial Refuge

The impact that different bioturbator species will have on seed settlement and seedling establishment will be a direct function of the magnitude and frequency of sediment disturbance. The former effect will be a function of the size of the bioturbator species and the location of its movement in the sediment horizon. The latter effect will likely depend on the density or actual mobility of the bioturbator species. For example, observations of sand dollars foraging through sediment indicate that sand dollars in this and previous studies will disturb the upper 1-2 cm of a 50 m^2 area every 3-8 days at conservative densities (~1 sand dollar m^{-2}, this study and Yeo et al 2013) and daily at high densities (6 sand dollars m^{-2}, Forehead & Thompson 2010). These observations, combined with experimental results showing seed displacement, suggest sand dollars exhibit remarkable ability to disturb the sediment and dislodge or disrupt seeds on the sediment surface or shallowly buried in the sediment. In contrast, sea stars moved at a faster rate and covered greater areas but disturbed less of the sediment profile than sand dollars and heart urchins. At their highest recorded densities, sea stars would move over the entire surface four times faster than sand dollars. At this level of activity, sea stars would frequently encounter seeds on the surface, but, should the seeds become buried, the seeds would largely escape the potential for sea star disturbance. During mating or stress behavior, however, sea stars regularly bury themselves in the upper 2 cm (Keesing et al. 2001, Lawerence et al. 2011). This behavior could move, overturn, or even bury recently settled seeds. Because *A. angulatus* (the sea star observed in Cockburn Sound) breeds *en mass* in late spring and early summer concurrent with *P. australis* seed release, the potential for this behavior to influence seed disturbance is substantial. The potential

for seed disturbance from sea stars may thus stem not from the rate of movement, but rather with movement associated with mating activity in the vicinity of recently settled *P. australis* seeds.

Although much slower than sea stars and sand dollars, heart urchins were buried entirely within the sediment as they moved and thus demonstrated more sediment turnover and seed disturbance. The heart urchins ploughed through the upper 4 cm of sediment, pushed seeds through the sediment, unearthed seeds from the sediment and even buried seeds originally on the surface, demonstrating a distinct capacity to disturb seeds. Estimates of urchin density and movement in this study indicate urchins will take substantially longer to disturb the same equivalent area as sand dollars or sea stars, but the impact of their movement on seeds on the surface or buried will be more substantial. Interestingly, related Spatangoida urchins have been reported to burrow deeper (5-15 cm) and move between 0.03 and 0.1 m day^{-1} (Buchanan 1966, Lohrer et al. 2005) suggesting urchin species could be even more disruptive to seedling recruitment than recorded in this study.

Implications of a potential seedling recruitment bottleneck from bioturbators

Here, we found bioturbator disturbance from three echinoderm detritivores can be a significant potential bottleneck to seedling establishment and successful seedling recruitment. For clonal seagrass species, seedling establishment is most likely to occur in unvegetated or sparsely vegetated sediment areas (Orth et al. 2006a) that are, in many locations around the world, also habitat to a diverse array of sediment bioturbators. Other phalanx seagrass species, like *P. australis*, may experience a similar bottleneck as seedling recruitment is likely highest away from adult clones, and within unvegetated

sediments where bioturbators are most likely to have their greatest influence. In these areas the high abundance and mobility of benthic fauna that are obligate bioturbators of bare substrate are a threat to newly settled seagrass seeds and therefore seagrass recovery via seedling recruitment. Diebacks of seagrass populations also generate bare sediment available for seedling recruitment (Orth et al. 2006c) and bioturbator foraging grounds. Bioturbator disturbance of seeds may thus slow the natural recovery of seagrass populations into these denuded areas. The variability of the bioturbator community captured in this study suggests the effect of these animals on seedling survival will vary in space and may be stronger in locations with bioturbators impacting deeper portions of the sediment. Because seeds provide seagrass populations important opportunities to disperse and to recover from disturbance (Kendrick et al. 2012, 2017, Jarvis & Moore 2010, Jarvis et al. 2014), incorporating the potentially additive effects of sediment bioturbator disturbance to seedling mortality may be important in predicting the distribution, stability, and recovery of seagrass populations. In addition, bioturbators have been shown to impact both seed and transplant based restoration (Davis et al. 1998) and should be incorporated in future restoration planning. A diverse and global community of bioturbators may thus impact seed settlement and seedling survival for seagrass species with diverse life histories in both natural and restoration settings.

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Figures and Tables

Figure 1:

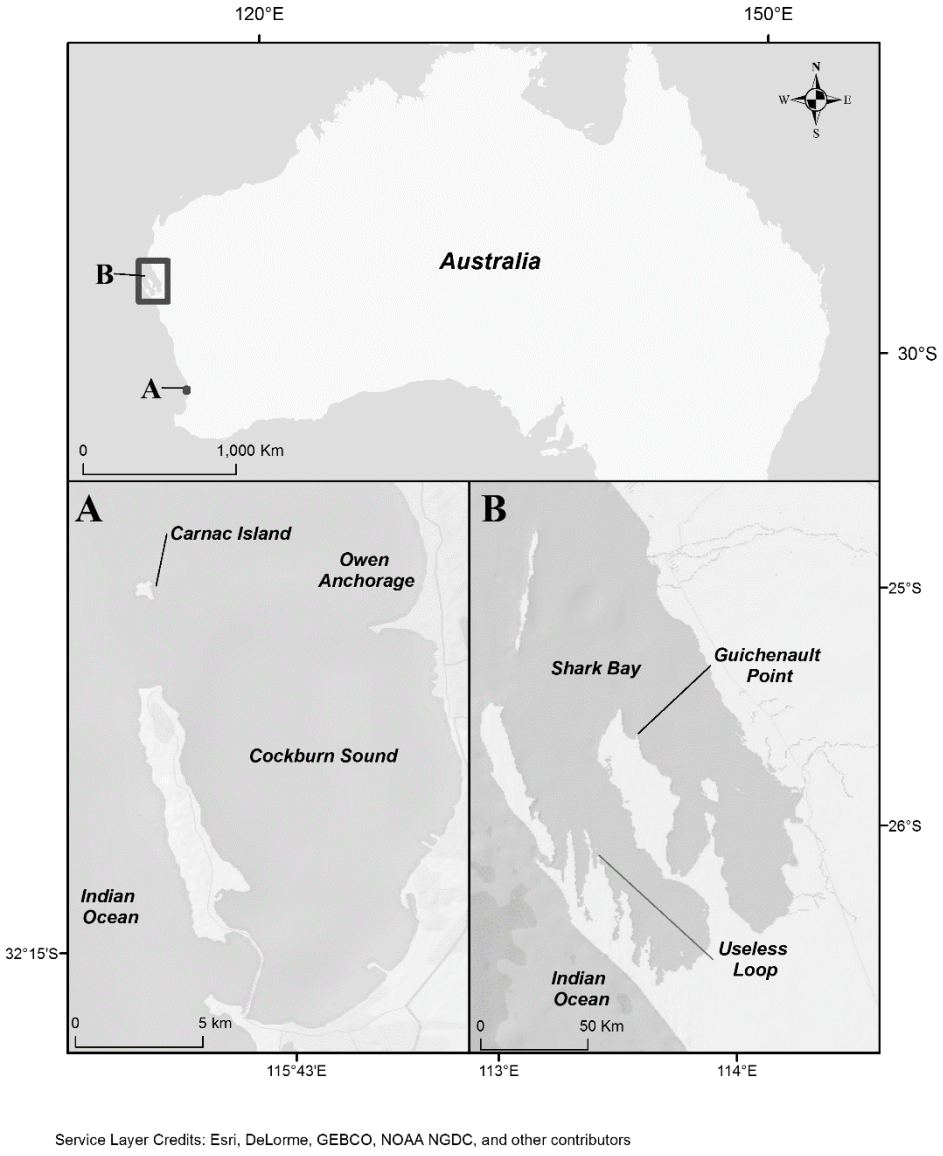

Figure 1. The survey and experimental locations along the west coast of Australia. Panel A shows locations of surveys and experiments in Cockburn Sound. Panel B shows locations of surveys and experiments in Shark Bay.

Figure 2

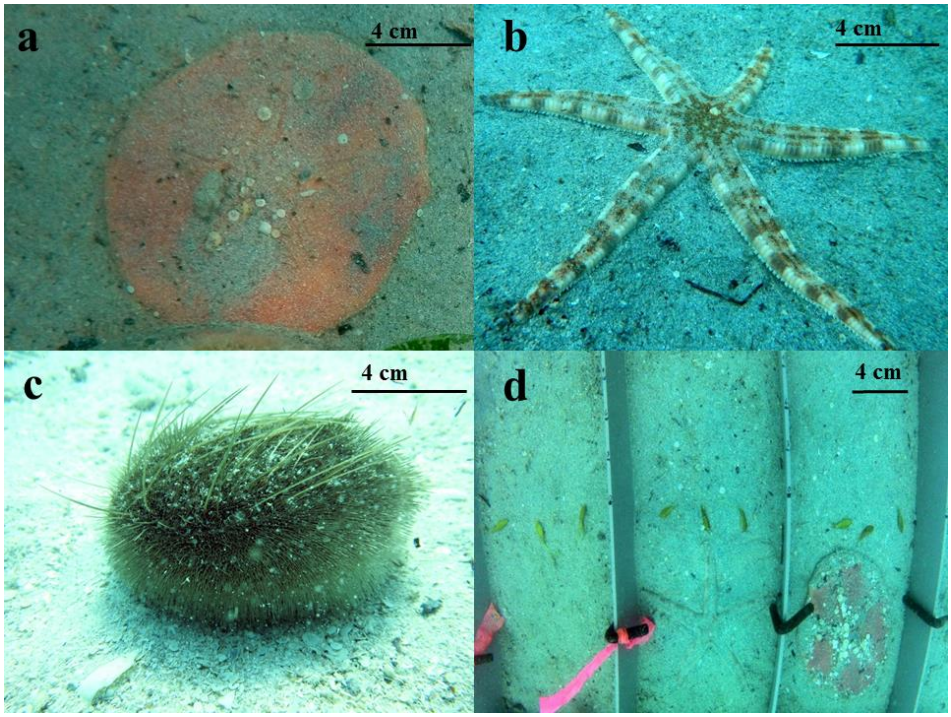

Figure 2. The sediment bioturbators common in surveys in Cockburn Sound and Shark Bay as well as the experimental lanes used to guide each of these animals into *Posidonia australis* seeds: (a) the sand dollar, *Peronella leseuri*; (b) the sea star, *Archaster angulatus*; (c) the heart urchin, *Breynia desori*, and (d) a sand dollar moving into *P. australis* seeds placed on the sediment surface in its path.

Figure 3:

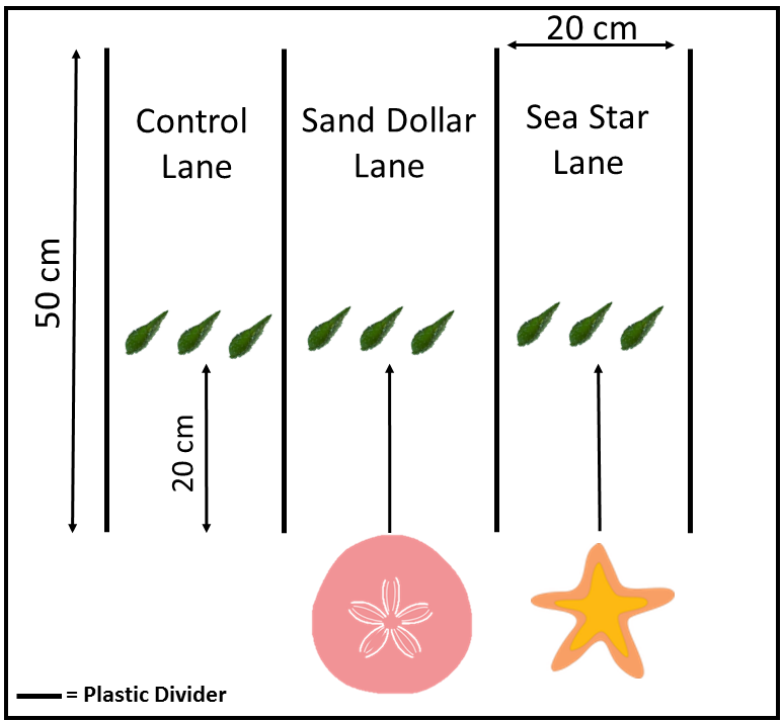

Figure 3. A schematic of the *in situ* experiments guiding bioturbators suspected of impacting seed settlement and seedling recruitment. Plastic dividers (7.5 cm H X 50 cm L) were inserted into the sediment 20 cm apart to generate three "lanes." Three seeds were then placed 20 cm down the length of each lane on the sediment surface (picture). A sediment bioturbator was introduced at the base of each lane, 20cm from the seeds, and guided into the seeds placed in their path. If the animals impacted any seed as they moved, the interaction was categorized as "disturbed." If no seeds moved over the course of the interaction, the interaction was categorized as "undisturbed." This experiment was repeated with three seeds buried 1cm into the sediment and later with one, one year old seedling placed 20cm down the length of each lane. Cartoons were sourced from the Integration and Application Network (Kleine 2010) and www.clker.com.

Figure 4:

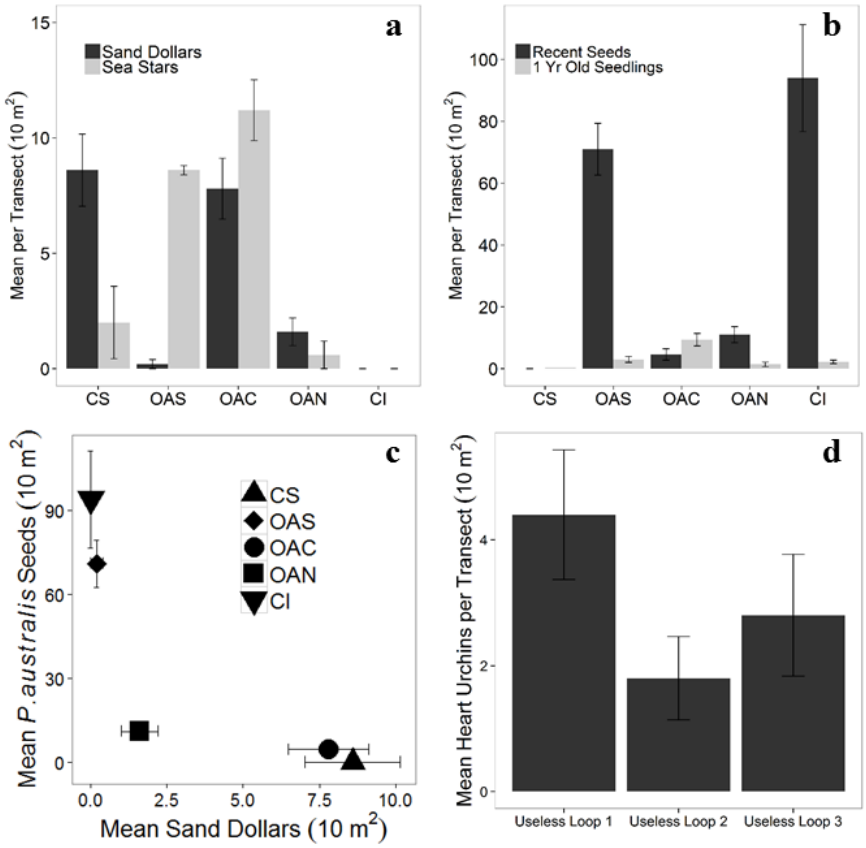

Figure 4. (a)The mean number of sand dollars and sea stars (± SE) at survey sites around Cockburn Sound. (b) The mean number of seeds and seedlings (± SE) surveyed at sites in Cockburn Sound. (c) The relationship between the mean density of sand dollars at a surveyed site and the mean density of seeds observed at that site. (d) The mean number of heart urchins (± SE) in Shark Bay present at Useless Loop. Heart urchins were absent from Guichenault Point. Labels: CS= Cockburn Sound, OAS=Owen Anchorage South, OAC= Owen Anchorage Central, OAN = Owen Anchorage North, CI = Carnac Island.

Figure 5:

Figure 5. Sand dollars placed in tanks covered in ~1-2 cm of fine sediment. The area over which sand dollars disturbed was monitored every six hours over 24 hours.

Figure 6:

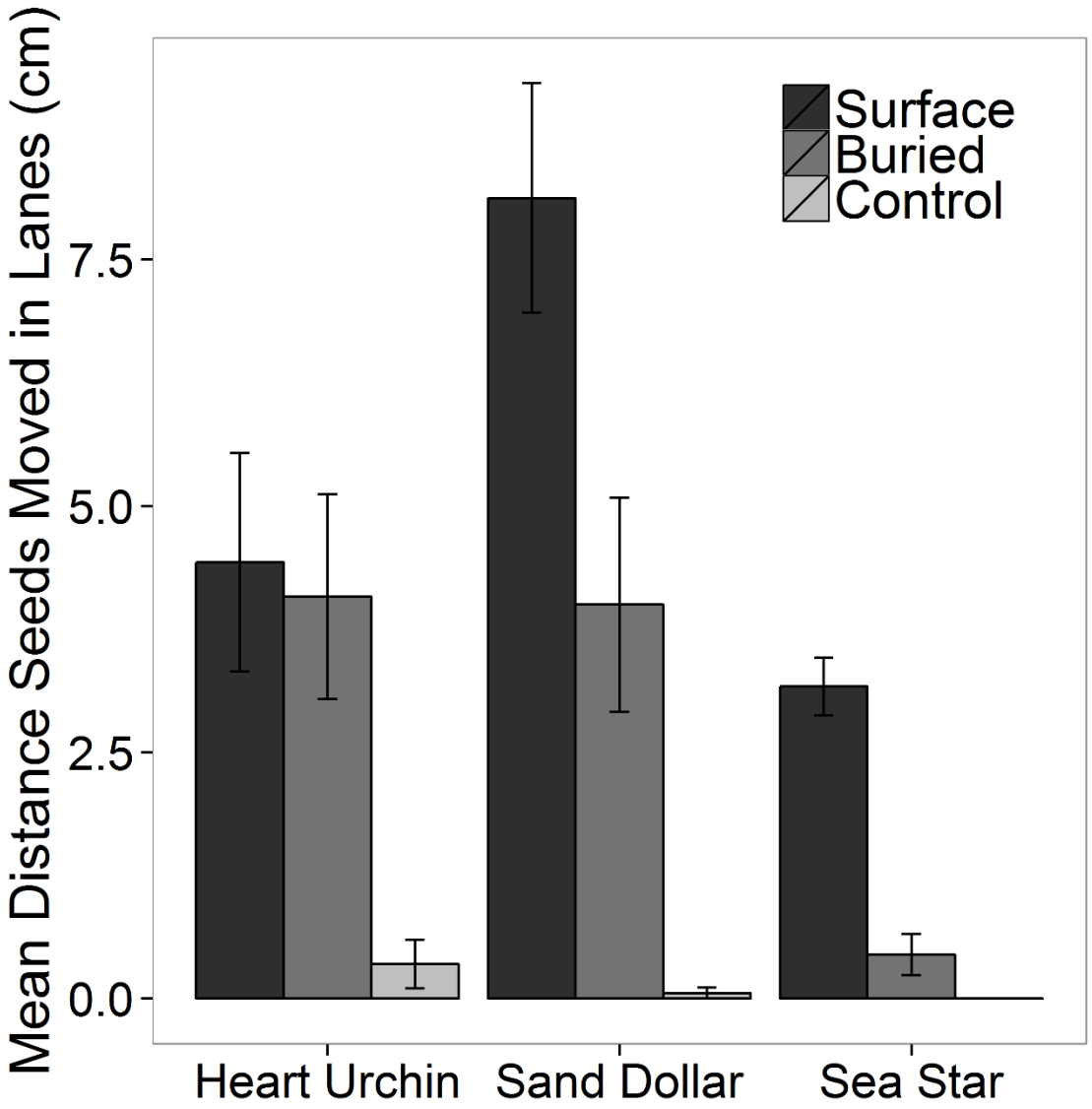

Figure 6. The mean (± SE) distance seeds moved in animal and control lanes (n = 9 lanes, each containing 3 seeds) for seeds on the surface or buried.

Table 1

Table 1. Spearman's rank correlation coefficients between the mean sand dollars or sea stars at a surveyed location and the mean number of *P. australis* seeds and seedlings observed at that location.

Bioturbator	Variable	S	rho	df	p
Sand Dollars	Seeds	40	-1	3	0.02*
	Seedlings	26	-0.3	3	0.7
Sea Stars	Seeds	30	-0.5	3	0.4
	Seedlings	8	0.6	3	0.6

*** = p < 0.05**

Table 2:

Table 2. A summary table for T-Tests evaluating differences in the mean movement of seeds placed on the sediment surface or buried 2 cm in lanes with or without bioturbators.

Seed Location	Variable	$\mathbf{mean}_{\mathbf{animal\ lane}}$	$\mathbf{mean}_{\mathbf{control\ lane}}$	*t*	Df	p
Surface	Sand Dollar Presence	8.1	0.1	9.5	7.2	< 0.001
	Sea Star Presence	3.2	0.0	5.2	3	0.01
	Heart Urchin Presence	5.0	0.6	-2.6	10	0.02
Buried	Sand Dollar Presence	4.0	0.0	3.1	8	0.01
	Sea Star Presence	0.4	0.0	1.8	8	0.10
	Heart Urchin Presence	4.0	0.0	-3.2	8	0.01

CHAPTER TWO

Herbivory regulates the establishment of native submerged aquatic vegetation (SAV) in a tidal estuary of the USA

Abstract

Herbivores are a diverse group of fauna that shape the distribution and composition of plant communities. In some cases, herbivory may prevent the re-establishment of submerged aquatic vegetation (SAV), such as *Vallisneria americana,* into systems. The goal of this study was to investigate the role and nature of herbivory on *V. americana* propagules with camera and transect surveys of grazing intensity and with field and laboratory grazing experiments using a suspected herbivore, the blue crab, *Callinectes sapidus*. Camera surveys recorded blue crabs clipping and consuming shoots of *V. americana* for the first time. Grazing intensity surveys in low salinity regions of the lower Chesapeake Bay indicated the majority of *V. americana* propagules (50-75%) were clipped off at their base within one week of planting. Field and laboratory experiments demonstrated blue crabs clip and consume *V. americana* as well as other rapidly colonizing, non-native SAV. Analysis of blue crabs caught in SAV beds in the Chesapeake Bay revealed SAV comprised 16 % of their stomach contents, suggesting low levels of blue crab SAV herbivory occurred over a wide area. Blue crabs are yet another animal on a growing list of animals documented to consume SAV for some portion of their diet. These results also suggest herbivores and omnivores, including the blue crab, can serve as an initial bottleneck to recovery of some SAV, like *V. americana*, but may not to other SAV species with, or under conditions where, rapid plant growth or high recruitment levels may overcome this grazing pressure.

KEYWORDS: plant population, non-native, restoration, recovery, blue crab

Introduction

Herbivores can influence plant community structure in both terrestrial and aquatic environments and subsequently the ecosystem services they provide (Cyr & Pace 1993; Burkepile 2013, Van Donk & Otte 1996; Green et al. 1997; Maron & Crone 2006). For a plant population to establish and persist in the presence of a robust herbivore community, it must develop mechanisms to withstand the grazing pressure in the system (Lodge 1991; Bakker et al. 2016; Scott et al. 2018). While the effects of herbivory on colonizing plant propagules may not initially be as evident as the effects of herbivory on established plant populations, the consumption of vulnerable, colonizing plant life history stages has long been hypothesized as especially important to the recruitment and dynamics of plant populations (Janzen 1970 & 1971; Harper 1977). For example, in a meta-analysis of seedling mortality, herbivory was the most frequently recorded source of seedling mortality across plant species (Moles & Westoby 2004). For clonal submerged aquatic vegetation (SAV) populations, consumption of propagules that are important for both developing new populations or maintaining existing populations may be an important bottleneck to population growth or recovery (Rybicki et al. 2001; Eriksson & Ehrlen 2008; Orth et al. 2012).

Wild celery, or *Vallisneria americana* (Michx), is a submerged angiosperm found in tidal and non-tidal freshwater habitats throughout North America and is widely consumed across this range by turtles, waterfowl, and crayfish (Lodge & Lorman 1987; Lodge 1991; Sponberg & Lodge 2005). *V. americana* is a meadow-forming species that grows long ribbon-like leaves from shoots near the sediment surface. As a dioecious, clonal plant species, *V. americana* individuals are capable of both sexual and asexual

reproduction (Sculthorpe 1967). Female flowers of *V. americana* are fertilized at the water surface and eventually produce fruits, each capable of dispersing 100-300 seeds (Lokker et al. 1997; Jarvis & Moore 2008). Individual shoots of *V. americana* reproduce asexually through stolon production and in northern habitats produce over-wintering buds. Both asexual and sexual reproduction are thus potentially important in the persistence, expansion, and recovery of *V. americana* populations.

Within the tidal freshwater and oligohaline regions of the Chesapeake Bay estuary, watershed inputs of nutrients and sediments in the 20th century lowered water quality and substantially reduced SAV populations (Moore et al. 2000; Cercro & Moore 2001; Kemp et al. 2005). In one region, encompassing the upper areas of the tidal James and Chickahominy Rivers, these nutrient and sediment loadings resulted in dramatic declines in native SAV, including *V. americana* (Moore et al. 2000). Areas historically vegetated with *V. americana* and other native SAV remain either unvegetated or are now colonized with mixtures of non-native vegetation such as *Hydrilla verticillata* (L.f. Royle) or *Najas minor* (All.) (Orth et al. 2017). Because *V. americana* has a wide salinity tolerance, 0 – 15 (Doering et al. 2001; Martin & Valentine 2012), and was historically abundant in the estuary throughout this salinity range, it has been the focal species for SAV restoration within the tidal freshwater and oligohaline environments of the James and Chickahominy Rivers. These controlled restoration attempts using both single adult shoots and seedlings in transplant garden plots have, to date, been largely unsuccessful. Restoration failure has been attributed to aquatic herbivory of unprotected propagules (Moore et al. 2010). In contrast, adult plants and seedlings of *V. americana* survived and grew within enclosures protecting *V. americana* from potential herbivores (Meier 2002;

Moore et al. 2010). These results point to herbivory as the critical bottleneck to *V. americana* recruitment and recovery within the tidal James and Chickahominy Rivers.

The goal of this study was to better understand the specific nature and role of herbivory limiting the re-establishment of this native, freshwater plant species into its original habitat. Specific objectives were: 1. To identify the primary herbivores consuming *V. americana* shoot propagules within the system; 2. To determine the grazing intensity of the herbivore community on individually planted *V. americana* propagules; 3. To evaluate the grazing intensity of a suspected generalist omnivore, the blue crab, *Callinectes sapidus*, on *V. americana* relative to a non-native SAV species present in the system; and finally, 4. To identify the diet of blue crabs within the James and Chickahominy River system to determine if vegetation was present in the diet of blue crabs in the natural system similar to blue crabs used in experimental trials.

Methods

Study Design

The study was conducted over two consecutive years, 2016 and 2017. In late summer (August-October) 2016, a field survey using underwater photography was conducted to identify potential *V. americana* herbivores adjacent to restoration plots in the James and Chickahominy Rivers, in the lower Chesapeake Bay, VA. In addition, *V. americana* vegetative propagules (shoots) were transplanted along transects over three trials to evaluate grazing intensity after 1 and 7 days at these same locations. After analyzing and interpreting the results from these surveys, *in situ* caging experiments were

conducted in 2017 to specifically evaluate the grazing effects of the blue crab, *Callinectes sapidus*, on transplants of *V. americana*. The blue crab was chosen as it was the only herbivore observed both during these surveys and in another previous study of SAV herbivory conducted in this region (Meier 2002). Additional laboratory experiments were then conducted to compare blue crab consumption between *V. americana* and a non-native species, *Hydrilla verticillata*, which is present and abundant in the tidal, freshwater and oligohaline portions of Chesapeake Bay. Last, blue crabs were collected outside experimental sites in the lower Chesapeake Bay to identify their diet preferences outside an experimental setting. Nursery grown vegetative propagules were used in all experiments. Prior research (Moore et al. 2010) at the sites noted here showed vegetative propagules and seedlings were consumed equally allowing us to use the vegetative propagules as a proxy for seedlings. Before transplanting, all *V. americana* and *H. verticillata* individuals were scraped clean of any obvious epiphytes. All applicable institutional and national guidelines for the care and use of animals were followed.

Study Sites

Locations in the James (37.310699, -77.155512) and Chickahominy Rivers (37.263984, -76.873465), VA were chosen because they historically supported stable SAV populations and are both locations of largely unsuccessful *V. americana* restoration efforts (Figure 1). Sites within the James River currently have no persistent SAV while sites within the Chickahominy River have fringing and seasonally persistent meadows of two non-native SAV species, *Najas minor* and *Hydrilla verticillata*. Field surveys, transplant herbivory surveys, and *in situ* caging experiments were conducted at depths $\leq$ 0.5 m MSL at these sites.

Herbivore Identification

A field survey using underwater photography was conducted in the James and Chickahominy Rivers, in late summer 2016 to identify herbivores most likely consuming *V. americana* transplants and seedlings. Four GoPro® cameras set to photograph at one second intervals were deployed 8 cm from 3-4 *V. americana* shoots on 11 separate occasions for ~2 hours. Due to camera malfunctions, obstructions to the field of view, and poor visibility, the duration of usable photography from a camera deployment varied between sampling events. This survey was conducted on three separate occasions in the James River for a total of 24 hours of footage. Within the Chickahominy River, the survey was conducted on eight separate occasions for a total of 54 hours of footage. More cameras were deployed in the Chickahominy River after determining photographs in this area were consistently and reliably of higher quality than at the James River location, and the observed clipping of shoots ~2 cm above the meristem suggested that the same herbivore was present at both locations. All recordings were conducted on rising tides (~half an hour after low) in case the herbivore was more active in deeper water. Photographs were inspected for any interactions, or physical engagement, with *V. americana* shoots. The total number of animals in the field of view and the number of animals directly interacting with (identified as touching, damaging, clipping, or biting) the transplants in the photographs were counted and identified to determine the most likely *V. americana* consumers.

Grazing Intensity

To quantify the intensity of *V. americana* consumption within the James and Chickahominy Rivers, one shoot of *V. americana* with at least 10 cm leaves was planted every half meter along ten meter un-vegetated transects at each location. All transplants were then inspected for herbivory after 1 and 7 days. In total, 20 shoots were planted at each site along each transect. A 10 m guide rope was laid between two PVC stakes with marks every 0.5 m to indicate a transplant location. Transplants were planted at ~2-3 cm within the sediment. After planting, the composition and percent cover of SAV within a meter of the planting line was determined every meter. Transplants were considered grazed if they were clipped to ~2 – 4 cm height, the characteristic mark of the dominant grazer within these systems (Figure 2). Missing shoots were labeled as such to distinguish between transplants whose leaves had been clipped ("grazed") and those who may have been consumed or lost by other means ("missing"). This procedure was repeated for three separate trials at each location in 2016. An additional transect trial was placed within a densely vegetated *Najas minor* meadow (~95% bottom cover) in the Chickahominy River to gauge if herbivory occurred within existing SAV in the system. Three additional transect trials were conducted at the same location in the Chickahominy River in summer 2017 to test if grazing intensity varied at this location between 2016 and 2017.

To directly estimate the grazing intensity of a potentially important herbivore, the blue crab, on *V. americana,* five, circular 0.06 m^2 aluminum wire (2 mm diagonal mesh size) cages were used to contain individual blue crabs with two *V. americana* transplants for 72 hours *in situ* in the Chickahominy River (Figure S1). An aluminum wire cage not containing a blue crab and an uncaged control, each also containing two *V. americana* transplants, were constructed adjacent to each caged crab treatment to form a block

containing one experimental unit of each treatment. Each transplant was cut to 20 cm height, and the number of intact leaves was counted. The location of each transplant within the cage relative to shore was also recorded to track consumption of each transplant in each cage. Blue crabs ranged in carapace width (CW) from 2.5 – 17.5 cm. Cages were constructed with aluminum wire (height = 40 cm) attached to plastic cylinder (height = 15 cm) with a 48" cable tie. At deployment, the plastic cylinder was pushed 8 cm into the sediment to prevent blue crabs from burrowing out or into treatments and anchored in place with one, 2 cm PVC and one rebar stake. After 72 hours, the height of all transplant leaves was measured and each leaf was inspected for bite marks. Physical damage to cages and the availability of blue crabs caught within unbaited crab pots resulted in uneven blocks of treatments between trials. In the end, data were collected from thirty-one blocks containing all three treatments over the eight trials. These *in situ* cages excluded other potential herbivores from *V. americana* transplants but provided alternative food items, such as epifauna in the water column and infauna within the sediment, for blue crabs within the cages. As a result, at the end of a given trial, cages were also visually inspected for any obvious alternative prey inhabiting them. Blocks of cages were placed into bare sediment in between clumps of the non-native, freshwater plants *Najas minor* and *Hydrilla verticillata*, which are prevalent in the system. Five, 0.07 m^2 sediment cores were taken and five, 2.5 m^2 dip net pushes (2 mm diameter mesh) were made within a *Najas minor* meadow adjacent to the experiments to estimate sediment infauna and epifauna in the *Najas minor* meadow surrounding the cage experiment. In addition, five, 20 cm W x 80 cm L mesh (500 μm) epifaunal bag samples (similar to Duffy et al. 2015) were taken from *N. minor* patches in between the blocks of

cages to further categorize the epifaunal community in the area. All epifaunal bag samples were emptied into plastic bags and frozen until contents could be identified in the lab.

Non-native SAV Consumption

To gauge if blue crabs consume non-native SAV present in the system at a similar rate to the native plant *V. americana*, blue crabs were collected from the Chickahominy River on eight occasions and placed in tanks with propagules of either *V. americana* or *H. verticillata* for 72 hours. Eight 100 L tanks filled to 25 cm were placed into an 1800 L tank filled with recirculating water chilled to 24ºC. Four vegetative propagules of *V. americana* or *H. verticillata* were planted in each 100L tank. The number of *V. americana* leaves or *H. verticillata* shoots was counted for each propagule and the height of all propagules was cut to 20 cm before planting. A single crab was introduced into two separate 100L tanks, each with four shoots of *V. americana*, and a crab was introduced into two separate 100L tanks, each with four shoots of *H. veticillata*. The remaining four 100L tanks, two tanks per plant species and each containing four propagules of the respective species, received no crabs and served as crab-less controls. Twenty-four hours after introducing crabs, the plant propagules were inspected and any uprooted propagules were replanted as any uprooting over this time period may potentially have resulted from crab acclimation to the tank environment. Seventy-two hours after introducing crabs into the tank, the height of each remaining leaf/shoot on a propagule was measured. *V. americana* leaves were also inspected for signs of tearing or biting. Suspected marks were categorized as "minimal" (> 1 mm but < 10 mm) or "heavy" (>10 mm). Four trials were conducted with "large" blue crabs (CW > 8 cm) collected with un-baited crab pots,

and four trials were conducted with smaller blue crabs (CW $\leq$ 8 cm) collected with a 50 cm mouth dip net (2 mm diameter mesh). Blue crabs ranged in size from 2 – 16 cm CW. In addition, at the end of each experiment crabs larger than 3 cm were removed from tanks and frozen for gut analysis to verify consumption of plant material had occurred.

Blue Crab Diet Survey

Dietary patterns were determined from crabs collected by seining at two locations on either side of the experimental area at the mouth of the Chickahominy River, as well as across from restoration plots at Westover Plantation in the James River. Sampling occurred from July to September, 2017, on five occasions during the peak biomass of SAV in the region (Moore et al. 2010). Two replicate seines (30 m L x 1.2 m H, with 0.64 cm mesh) were made at each site during each sampling round. Each replicate seine was pulled over the same area but was separated by a minimum of 30 minutes. For each seine pull, the net was pulled out perpendicular to shore until fully extended or a depth of 1.2 meters was reached, at which point the offshore end of the seine was pulled down-current back to shore.

All captured crabs were placed immediately on ice to reduce digestion of stomach contents until frozen. In the lab, the carapace width, sex, and any apparent damage to the crab were recorded before foreguts were dissected. The percent fullness of foreguts was then estimated as the displacement volume of a foregut when placed in either a 10 or 25 mL graduated cylinder filled with water, depending on the size of the foregut (see Seitz et al., 2011 for further discussion of methods). Each foregut was then emptied into a petri dish containing water and allowed to settle for one hour at which point the relative

contribution of amphipods, clams, copepods, crabs, gastropods, isopods, ostracods, polychaetes, shrimp, and plant matter to stomach fullness were estimated.

Statistical Analyses

Grazing Intensity

A generalized linear model (GLM) fit to a quasi-binomial distribution was constructed to determine if the location or time period after planting during a grazing intensity trial influenced the number of grazed transplants observed along transects in 2016. A separate GLM, also fit to a quasi-binomial distribution, was then used to compare the grazing intensity along transects at the mouth of the Chickahominy River between 2016 and 2017. The specific transect trial during which grazing was evaluated was included as an additive term in each model to account for any temporal variability associated with grazing intensity at each location over the course of the three survey trial periods. Models were fit to quasi-binomial distributions to account for any potential overdispersion within the observed data. Model fit was evaluated graphically.

A linear mixed-effects model was constructed to determine if the change in total leaf length for transplants in cages containing blue crabs was significantly different to the change in total leaf length for transplants in control cages without blue crabs or uncaged transplants exposed to the entire herbivore community after 72 hours. The trial during which a given set of treatments was evaluated and the block within which a cage was situated were considered as nested, random terms in this model to account for any random spatial or temporal differences in grazing at the sampling location. The difference in total leaf height response variable was square-root transformed to meet model

assumptions. Post-hoc Dunnett's multiple comparisons of least square means were conducted to evaluate differences in change in total transplant leaf length specifically between transplants inside cages containing crabs and transplants planted outside cages. A generalized linear model was then used to establish if the estimated percentage of plant matter in a crab stomach was related to the difference in transplant leaf height within a given cage.

Non-native SAV Consumption

A linear mixed-effects model was used to compare the change in total height of *V. americana* or *H. verticillata* propagules in experimental tank systems with or without crabs after 72 hours. The presence or absence of a crab and the species of SAV present in the tank were treated as interactive terms in the model while the size of the blue crab added to the tank during a trial was considered a separate fixed factor. The individual trial in which a crab was introduced to tanks was treated as a random factor to account for any variability resulting from successive trials. Categorical classifications of bite marks were analyzed with odds ratios to determine if the odds of observing tear or bite marks on *V. americana* differed between tanks with and without crabs. Fisher's exact tests were then used to estimate if the observed frequencies of tear or bite marks were significantly different than expected frequencies of marks (i.e. no difference in tearing or biting between crab and control tanks). A generalized linear model was then used to establish if the estimated percentage of plant matter in a crab stomach from a given tank was related to the difference in leaf or shoot height within that tank.

A type I error rate of 0.05 was established for all statistical tests. Generalized linear models and linear mixed-effects models were built with the *glm* and the *lmer* function from the *lmerTEST* R package (Kuznetsova et al. 2014). Post-hoc Dunnett's multiple comparisons of least square means were conducted with the *contrast* function in the *lsmeans* package (Lenth 2015). All statistics were performed in R statistical analysis software (R Development Core Team 2015).

Results

Identifying Herbivores

Similar species assemblages were recorded in the Chickahominy River as in the James River. The most common species identified (Table S1) were tessellated darters (*Etheostoma olmstedi*), juvenile sunfish (*Lepomis* sp.), and blue crabs (*Callinectes sapidus*). Blue crabs were the only observed animals to interact with *V. americana* transplants (Figure 4(a)). Crabs interacted with transplants by grabbing leaves on six separate occasions, damaging transplants on two occasions by clipping leaves, and consuming a transplant leaf on one occasion (Video 1 shows the time lapse photography of this consumption).

Grazing Intensity

Significantly more transplants were consumed within seven days of planting than within one day of planting ($\beta = 9.3 \pm 1.7$, $P < 0.001$, Figure 3). On average, < 25% of the transplants were grazed after 1 day but 40 – 75% were grazed within seven days at both locations. No significant differences in transplant grazing were detected between

locations ($p = 0.1$) and no significant interaction term was detected ($p = 0.2$). Grazing intensity was significantly different among the three successive trials (Table S2). Similarly, grazing intensity over the duration of a trial interacted significantly with the year of sampling in the Chickahominy River ($\beta = 0.08 \pm 1.9$, $P < 0.001$, Figures S2 & S3). Although diagnostics of this generalized linear model describing grazing intensity between 2016 and 2017 suggest a poor model fit, data visualization corroborate model results (Figure S3) and generally suggest grazing occurred in both 2016 and 2017, but that the duration over which a transplant experienced this grazing differed between the two years. Regardless of the year or location, however, no transplants survived until the end of the growing season. At the end of the six successive sampling weeks in 2017, for example, only 3 of the 60 total planted shoots remained ungrazed (5%) and none survived. The additional transect placed within a *N. minor* meadow in 2016 exhibited similar herbivory trends to adjacent transects placed in sediment with lower *N. minor* cover, with 75% of shoots intact after 24 hours and only 30% remaining after 1 week (Figure S4). Grazing recorded along transects in 2017 also compliments this finding, as *N. minor* was present along previously bare sediment transects at the mouth of the Chickahominy River in 2017.

The change in heights of unprotected *V. americana* transplants ($\beta = 54 \pm 0.67$, $P < 0.001$) and transplants in cages containing one blue crab ($\beta = 25 \pm 0.67$, $P < 0.001$) were significantly different than the change in heights of transplants in control cages without crabs after 72 hours (Table 1, Figure S5). Dunnett's comparisons indicated significant differences in final shoot heights ($t_d = -2.9$, $df = 84$, $P = 0.009$) between transplants from cages containing crabs (Least Squares Mean CI: 4.4 – 7.8) and transplants outside any

enclosure (open controls) (Least Squares Mean CI: 6.8 – 10). Clipped transplants removed from *in situ* cages containing crabs appeared similar, however, to clipped transplants exposed to the entire herbivore community in the open water (Figure 2 (b) & Figure 4 (b)). No significant change in transplant heights was detected between cages containing large or small crabs ($p = 0.38$). Tassellated darters (*Etheostoma olmstedi*), mud crabs (likely *Rhithropanopeus* sp.), brackish water clams (*Rangia cuneata*), various amphipod species, and small juvenile blue crabs (~1 cm CW) were observed in crab and control cages. No significant relationship was detected between the difference in total transplant leaf length within a given cage to the estimated volume of plant matter in a blue crab's stomach after a cage trial ($P = 0.1$, Figure S6). Plant matter was, however, present in 17 of the 18 dissected blue crab stomachs and was on average 46% of the estimated stomach volume of caged blue crabs after 72 hours (Figure 5 (a) & (b)).

Non-native SAV Consumption

Propagules of *V. americana* and *H. verticillata* decreased significantly in height after 72 hours in tanks with blue crabs relative to propagules in tanks without blue crabs ($\beta = 44.7 \pm 1.44$, $P < 0.001$, Figure 6). No significant differences in the change in total shoot height were detected between tanks planted with different propagule species ($p = 0.6$). Tearing or bite marks were also more likely to be found on *V. americana* leaves in tanks with crabs (odds ratio: 10.5, 95% CI: 1.5-73, $p < 0.001$, Figure 4 (c) & S7) than in tanks without crabs. Although no formal categorization of tear or bite marks was conducted for *H. verticillata* shoots, *H. verticillata* shoots were stripped of leaves in tanks containing blue crabs on several occasions (Figure 4 (d)). The difference in total shoot height for a given tank was not significantly related to the estimated percentage of plant

matter in a crab's stomach ($\beta = 0.2 \pm 0.1$, $P = 0.05$, S8, & S9) and clipped shoots were observed floating within tanks (Figure S10).

Blue Crab Diet Survey

The majority of blue crab stomach volume (on average 44%) consisted of unidentifiable material. Plant matter was present in 32 of the 52 collected crabs (61%) and was the most prevalently identified food item, consisting of on average 16% of stomach contents (Figures 5 (c & d) & 7). Bivalves were the second most prevalent, identifiable food item, contributing on average 14 % of stomach contents.

Discussion

Our results provide an important example of how plant community structure and re-establishment, especially in an estuarine system, may be affected by herbivory of vulnerable, colonizing propagules that are important to the recruitment and dynamics of plant populations (Janzen 1970 & 1971, Harper 1977). We have demonstrated using field observations and field and laboratory experiments that the blue crab affects both native and non-native vegetation in the oligohaline waters of Chesapeake Bay by both clipping and consuming these plants. This behavior, which removes photosynthetic tissue from propagules, was found to occur consistently in both the James and Chickahominy Rivers. Grazing of this nature was previously demonstrated to prevent the initial recovery of one *V. americana* (Moore et al. 2010), but has not prevented the emergence and persistence of other non-native SAV species. The combined results of the current and previous studies suggest herbivory, likely from a generalist, marine omnivore, the blue crab, could act as the bottleneck to population recovery of a native species but not necessarily the

non-native species in the area. This outcome may be related to the higher reproductive, growth, and dispersal mechanisms of the non-native vegetation which allows them to persist despite herbivory.

Blue Crab Herbivory

This study is the first to document targeted consumption of submerged vegetation by an estuarine omnivore, *C. sapidus*, under experimental and natural settings. While variability in the prevalence of SAV in stomachs among the individuals observed here was large, these observations in combination with previous diet studies indicate the contribution of plant matter to blue crab diets could be 4-29% (Laughlin 1982; Alexander 1986; Wolcott & O'Connor 1992; Seitz et al. 2011). Previous studies have demonstrated blue crabs may derive nutritional value from vegetation (McClintock et al. 1991). Because blue crabs are ubiquitous and extremely common (they are one of the most valuable commercial fisheries in the Chesapeake Bay) in low salinity estuarine regions throughout their range (Posey et al. 2005; Seitz et al. 2003), they could play a role in regulating population dynamics of SAV and other plant populations both here, and in many other areas where they co-exist (Alexander 1986). Blue crabs may be yet another of a growing and diverse suite of animals, from sea urchins and sea turtles to deer and sharks, that can derive some portion of their diet from submerged aquatic vegetation (Thayer et al. 1984; Eklöf et al. 2008; Fourqurean et al. 2010; Ceacero et al. 2014; Leigh et al. 2018).

The clipping of single *V. americana* plants spaced at 0.5 m intervals from one another observed in this study suggests the blue crab may feed opportunistically on sparse shoots. Other known herbivores in the system, such as migratory waterfowl,

muskrat (*Onidatra zibethecus*), or red bellied turtles (*Pseudemys rubriventris*), may seek larger stands of vegetation which will provide them a higher foraging efficiency than isolated shoots (Spongberg & Lodge 2005). Crayfish also have been shown to clip and consume *V. americana* in freshwater habitats (Lodge & Lorman 1987), but none were observed in this oligohaline system. Although additional herbivores are likely present in the James and Chickahominy Rivers, their abundance and influence were not detected in this or previous studies (Meier 2002).

Observations of clipped but unconsumed leaf material floating within experiments, as well as clipped and heavily damaged leaves, support an opportunistic blue crab herbivory hypothesis, but also suggest blue crabs may "sample" SAV and then either partially or totally consume clipped plant material. Crabs in this study most commonly clipped leaves at their base and clipped every leaf from a shoot in most instances. Interestingly, some blue crabs in experimental tanks may have torn or bitten sections of leaves (Figure 4 c & d) without clipping the entire shoot or leaf at the base. These observations, the variability in the abundance of plant matter among blue crab stomachs, and the difference in clipping between transplant leaves in cages with one blue crab and transplant leaves exposed to the entire herbivore community offers evidence some blue crabs may consume SAV more than others. The size of blue crabs and other unexplored variables, for example alternative food availability, may explain the variability in blue crab vegetation consumption. Although epiphytes were initially removed from all vegetation used in experiments in this study, epiphyte growth could also lead to accidental grazing of SAV. The abundance of *M. leucophaeata* and other species both growing on vegetation and found within the stomachs of blue crabs collected

in the system (Table S3 & Figure S11 a & b) suggests incidental damage and consumption of SAV may occur and could explain damage to vegetation without consumption of the vegetation (Video S1). Despite the potential for blue crab scavenging for epiphytes to damage SAV, the photographic and diet observations in this study clearly demonstrate some crabs directly consume SAV.

Surprisingly, the non-natives *H. verticillata* and *N. minor* also appeared as a significant component of the blue crab diet (16%), in addition to epifauna and infauna found in these meadows, e.g. mussels (*Mytilopsis leucophaeata*), gastropods (*Lymnea* spp.), and amphipods (*Corophium* sp.). Our diet data reveal the value of these non-native SAV communities to blue crab populations within the oligohaline portions of the lower Chesapeake Bay, and possibly elsewhere where they occur.

Persistence of SAV with herbivory

Numerous studies in terrestrial and aquatic environments have shown that herbivores can alter the structure and composition of plant communities (Cyr & Pace 1993; Hanley 1998; Bakker et al. 2016). Our results in an aquatic environment demonstrate that blue crabs consume all studied SAV species, yet observations in the Chickahominy River found an abundance of *N. minor* and *H. verticillata* in the vicinity of experiments despite their documented consumption of these species. Indeed, much of the shallow water areas of the Chickahominy River and many other low salinity regions of the Chesapeake Bay maintain dense cover of these two species, and sometimes *V. americana,* despite the presence of blue crabs (Orth et al. 2017). The reproductive potential and dispersal characteristics of each SAV species, the presence of water quality conditions suitable for rapid SAV growth and expansion, and the foraging behaviors of

herbivores, such as the blue crab, may help to explain the composition of SAV communities in the James and Chickahominy Rivers.

All three SAV species reproduce sexually, producing large numbers of seeds, and asexually, through rhizome or stolon extension (Langeland 1996, McFarland & Shafer 2008, Les et al. 2015). Propagule production and supply, however, differ among the three. For canopy-forming species, such as *N. minor* and *H. verticillata*, vegetative fragments clipped or ripped away from the parent plant are often shoots that can disperse and re-root to colonize new habitat (Rybicki et al. 2001). In many cases the clipping or cutting of *H. verticillata* shoots has been found to only temporally reduce their abundance and regrowth occurs rapidly (Langeland 1996). However, for *V. americana*, a meadow forming species whose leaves grow into the water column from a shoot in the sediment, clipped or torn vegetative fragments are often leaf material not capable of surviving and colonizing new habitats. Thus, herbivory, particularly from the blue crab, can generate new propagules of *N. minor* and *H. verticillata*, but not so with *V. americana*. As a result, herbivory of very sparse SAV could further suppress propagule production of *V. americana* compared to these other SAV species.

The presence of large, dense stands of *V. americana* in the upper Chesapeake Bay (Orth et al. 2017) and other areas despite the presence of blue crabs suggests *V. americana* populations can overcome herbivore pressure. Future research should explore whether blue crabs or other herbivores target SAV propagules in other systems (Figure S12) and whether the proximity, density, and diversity of SAV communities, additional propagule availability, or fluctuations in herbivore intensity allow establishing *V. americana* populations to overcome grazing pressure.

Conclusions

Our results demonstrate that blue crabs can consume SAV in small to moderate amounts as part of their diets in oligohaline environments. For some SAV species such as *V. americana*, herbivory, likely from the blue crab, could prevent population re-establishment in areas with low SAV propagule availability. Although we have shown that blue crabs also consume other SAV species, including *N. minor* and *H. verticillata*, the capacity of these SAV species to reproduce and spread rapidly using both seeds and vegetative propagules may allow them to colonize available habitats and overcome this grazing pressure limitation. Reductions to herbivore populations, increased propagule production and dispersal through restoration efforts (Orth et al 2012), and direct exclusion of herbivores from restored, founder beds (Moore et al. 2010) may all be necessary for some species populations to reach the size and abundance necessary to overcome herbivory bottlenecks and become self-sustaining.

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Figures and Tables

Figure 1:

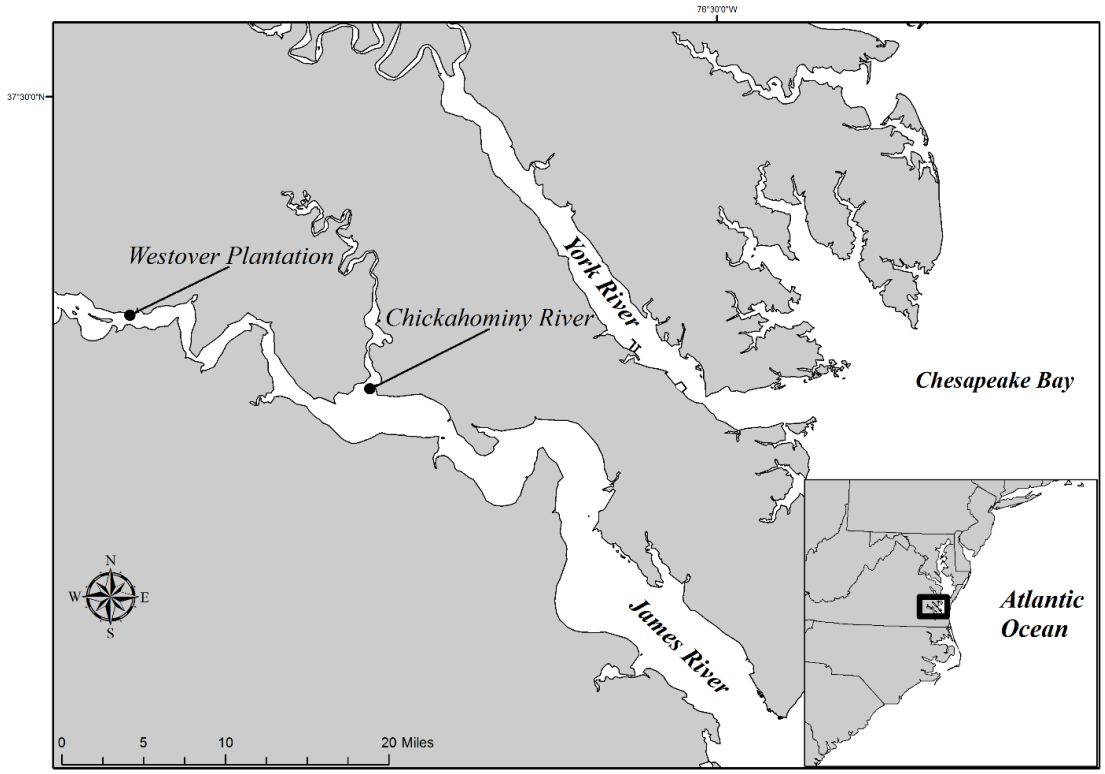

Figure 1. The location of experiments and surveys throughout the tidal, freshwater James River and Chickahominy River, Virginia.

Figure 2:

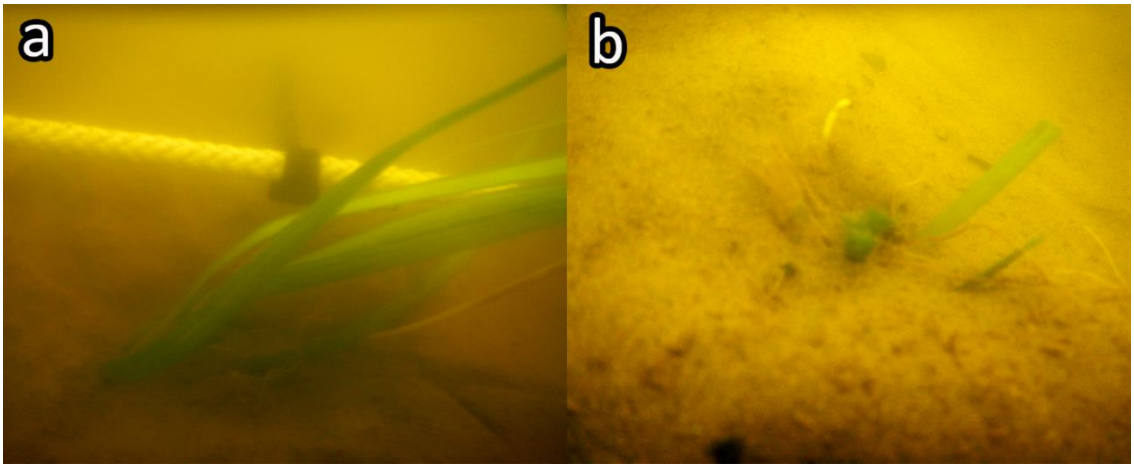

Figure 2. (a) A *V. americana* shoot planted every 0.5 m along the 10 m transect used for grazing intensity surveys in the James and Chickahominy Rivers. (b) a clipped *V. americana* shoot planted along a transect at the mouth of the Chickahominy River

Figure 3:

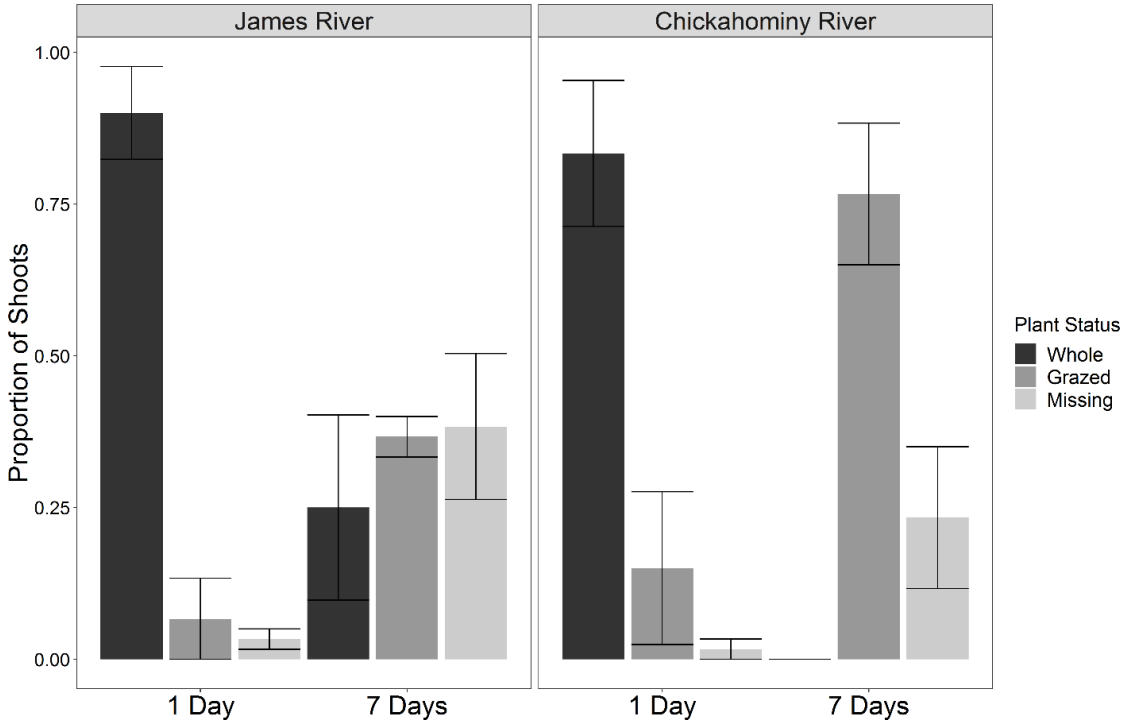

Figure 3. The mean proportion of shoots (± SE, n = 3 trials) whole and intact, grazed, or missing after one day and seven days along transects (20 shoots per transect) in the James and Chickahominy Rivers in late summer 2016.

Figure 4:

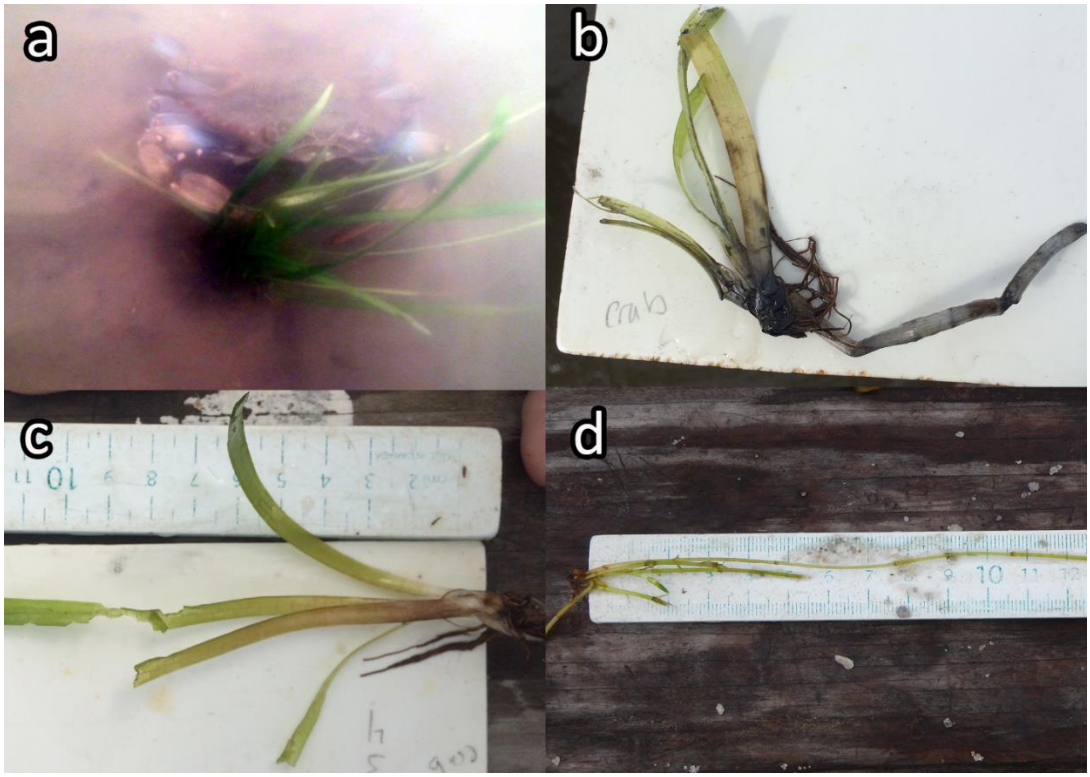

Figure 4. The signs of *C. sapidus* herbivory observed *in situ* in the James and Chickahominy Rivers and within laboratory experiments: (a) A *C. sapidus* photographed interacting with a *V. americana* transplant in the Chickahominy River; (b) a clipped *V. americana* shoot from a cage containing one *C. sapidus* from the *in situ* caging study conducted in the Chickahominy River; (c) a *V. americana* shoot with a bite mark categorized as "heavy" (> 1 cm); and (d) a shoot of *Hydrilla verticillata* removed from a tank containing one *C. sapidus*. All of the whorled leaves, normally 5 per node, have been stripped from the shoot and several shoots have been clipped.

Figure 5:

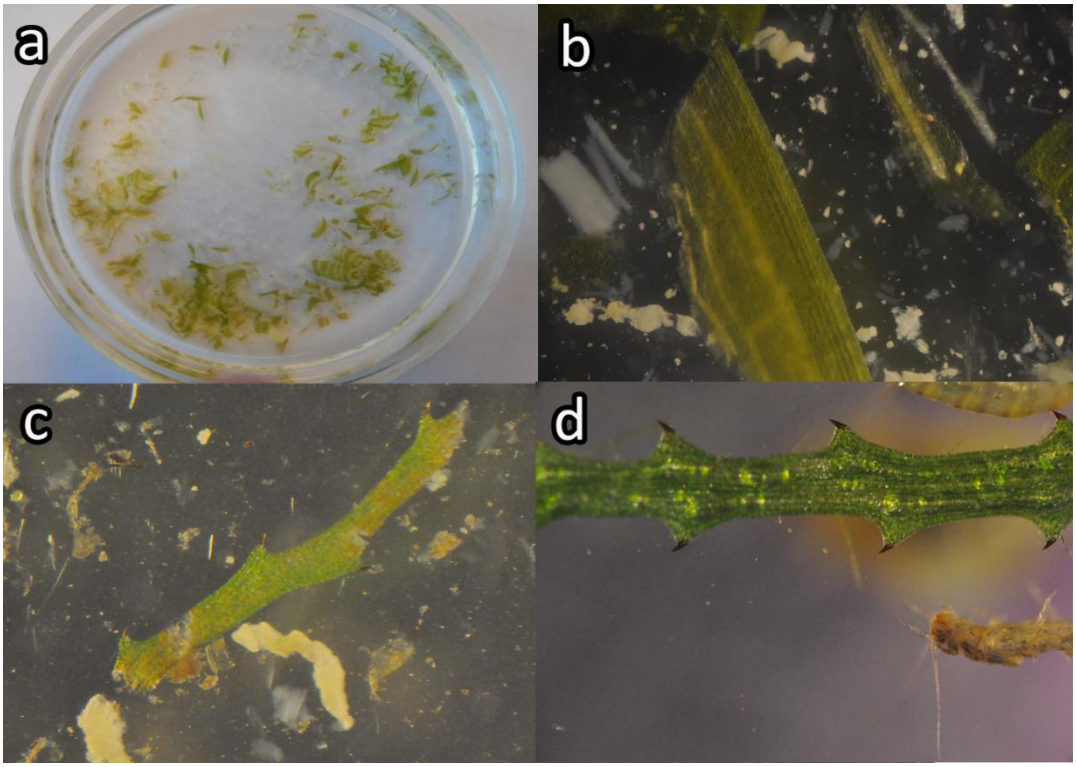

Figure 5. Plant matter within the stomachs of *C. sapidus*: (a) the stomach of a *C. sapidus* after 72 hours in a tank with 4 *Vallisneria americana* transplants; (b) a magnified imagine of *V. americana* pieces found in the stomach of a *C. sapidus*; (c) a piece of *Najas minor* found in the stomach of a *C. sapidus* collected within the Chickahominy River; and (d) a photo of a freshly collected piece of *N. minor*.

Figure 6:

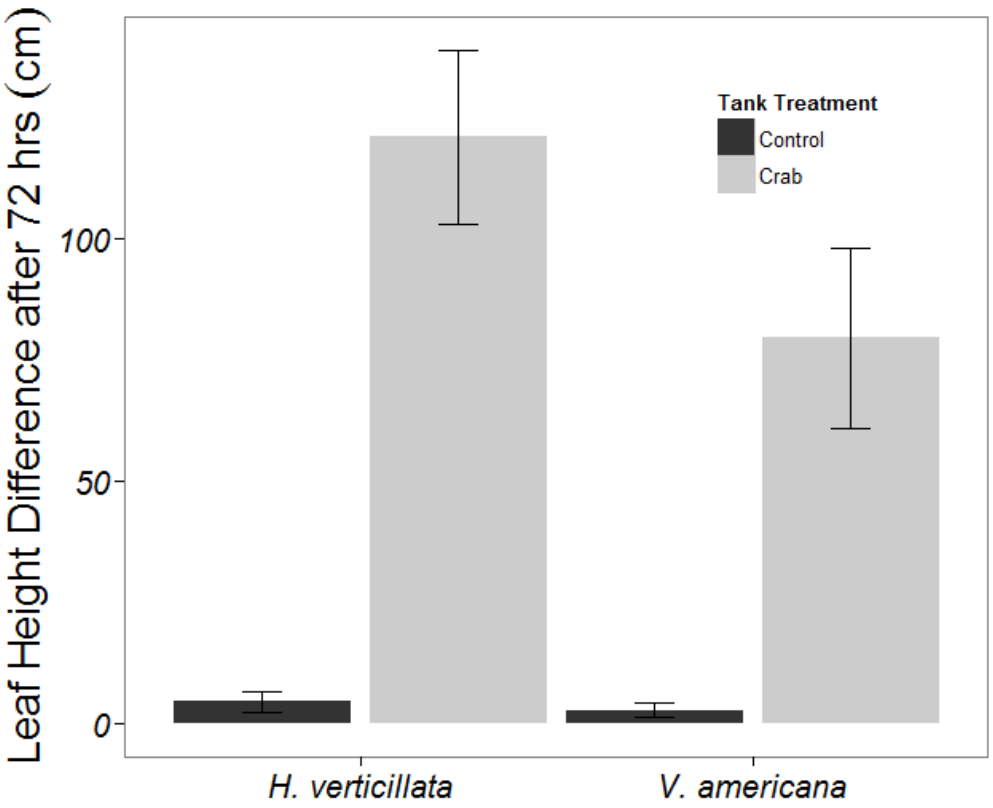

Figure 6. The mean difference in leaf heights per tank (± SE) for *H. verticillata* (n = 16) or *V. americana* (n = 14) after 72 hours with or without a *C. sapidus* in the tank.

Figure 7:

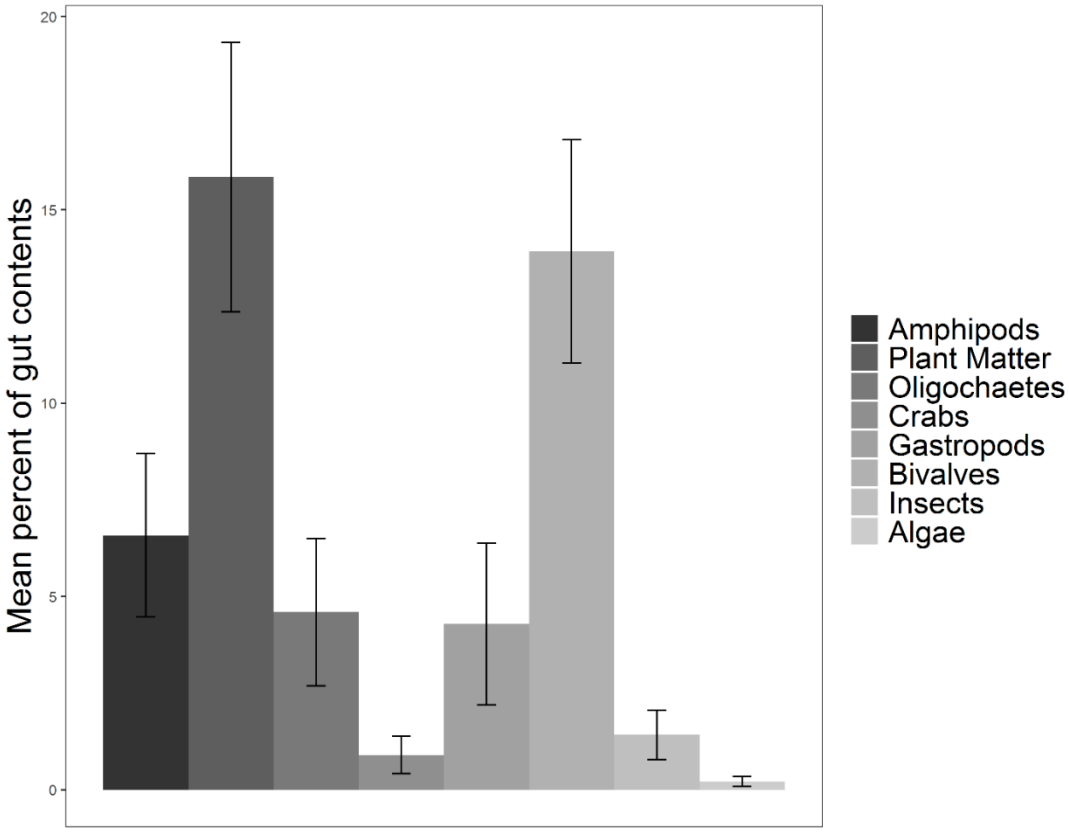

Figure 7. The mean percentage of identifiable food items (± SE) in the guts of *C. sapidus* collected from seine nets in the James and Chickahominy Rivers in 2017 (n = 52).

Table 1:

Table 1. A summary table for a linear mixed effects model fit evaluating differences in the length (cm) of *V. americana* shoots remaining after 72 hours in (caged control) and out (uncaged control) of cages and in cages with (crab) and without (caged control) *C. sapidus*.

Variables	**Variable Levels**	**Estimate**	**SE**	**df**	**p**
Crab Treatment	Caged Control	-	-	-	-	-
	Crab	25	0.67	83.9	**< 0.001****
	Uncaged Control	54	0.67	83.9	**< 0.001****
Crab Size	Small (≤ 8cm)	-	-	-	-
	Large (> 8 cm)	1.2	1.3	4.9	0.4

*** = p < 0.05**

**** = p < 0.001**

Video 1

See uploaded video of blue crab consumption

https://drive.google.com/file/d/1KVOhs0kMTQKb_zwIO6Hpit89VyXWQSqc/view?usp=sharing

CHAPTER THREE

The role of sexual reproduction in the maintenance of established Zostera marina meadows of Chesapeake Bay

Abstract

For clonal plants like *Zostera marina*, the role of sexual reproduction in the maintenance of populations can vary widely. Interactions between adults and seedlings within existing populations could threaten seedling survival and limit sexual reproduction in the vicinity of adult shoots. The goals of this study were to determine: 1. if *Z. marina* seedlings establish and recruit within existing *Z. marina* meadows of Chesapeake Bay, 2. if interactions between seedlings and surrounding adult shoots influence the survival of established seedlings. A three-year survey identified established seedlings within *Z. marina* meadows at peak biomass every year. Concurrent seed addition experiments suggested seed supply could influence seedling establishment rates. A survey tracking the survival of tagged seedlings, as well as the height and density of surrounding adult shoots, suggested adult shoots could negatively impact seedling survival. Experiments then demonstrated that seedlings without neighboring shoots survived longer than those with neighboring shoots. Last, two transplant garden experiments comparing the survival of plots with and without seeds suggested seedling recruitment is important to maintain bottom cover where disturbances generate gaps in the adult population. These results show that seedlings establish within the meadows of Chesapeake Bay, and that some survive to recruit into the adult population. Competition with existing vegetation is a potential factor compromising seedling survival. Sexual reproduction may thus most likely occur in, and be most important for, clonal plants that experience seasonal disturbance. Gaps in seagrass canopies and sediments away from existing vegetation may be environments favorable for seedling recruitment.

Introduction

The role of sexual reproduction in the population dynamics of a species can vary widely for clonal plant species, like seagrasses, capable of both sexual and asexual reproduction. Determining the pattern and relative importance of seedling recruitment, and therefore sexual reproduction, is critical to understand both the population dynamics and the life history evolution of these species (Erikkson 1989, 1993). Clonal species for which seedling recruitment is rare among adult plants exhibit "initial seedling recruitment" (ISR) patterns with high dependence on asexual reproduction after an initial seedling recruitment cohort. Seedling recruitment within a population is low or non-existent subsequent to this initial recruitment wave. Conversely, species exhibiting a "repeated seedling recruitment" (RSR) pattern consistently depend upon seedling recruitment among adult plants for population maintenance. These classifications may represent the extreme endmember classifications for designating the role of sexual reproduction for a given species, as populations may differentially rely on sexual reproduction for population maintenance and resilience where disturbance plays a role in breaking down space or resource limitation.

Because seagrasses produce both asexual and sexual progeny, their seedlings may potentially not only germinate among adult plants of the same or different species but also the asexual progeny, or ramets, of their own parent plant. The presence of adult plants surrounding seedlings may limit the space, light, nutrients, and other limiting resources available to seedlings and can therefore threaten seedling survival (Bullock 2000, Silvertown & Bullock 2003). For seagrasses, the density and height of surrounding shoots may dictate the extent to which adult shoots exploit resources more effectively

than seedlings in their vicinity (Robertson & Mann 1984, Bintz & Nixon 2001, Zimmerman 2003, Ralph et al. 2007). The density and height of seagrass shoots can, however, change with seasons and the availability of critical resources, such as light or nutrients (Orth 1977, Short 1983, Dennison 1987, Van Lent et al. 1995). Changes in the adult population structure could therefore dramatically alter the interactions between existing clones and seedlings in space and time, with high densities and heights of adult shoots likely providing the most severe resource competition with seedlings during peak growth and biomass.

Zostera marina is the dominant seagrass in the temperate waters of the Northern Hemisphere. Across the species' distribution, populations invest and rely variably on sexual reproduction for population maintenance and resilience. Populations with an "annual" life history subsist primarily on sexual reproduction and recruit from seed annually at locations where populations collapse seasonally (Robertson & Mann 1984, Santamaria-Gallegos 2000; Jarvis et al. 2012; Kim at al. 2014). Sexual reproduction, through dormant seeds, allows these populations to re-vegetate areas once environmental conditions have improved. Conversely, populations with a "perennial" life history rely substantially less or not at all on sexual reproduction and largely survive through asexual reproduction (Reusch et al 1999, Billingham et al. 2003). Asexual reproduction can be less energetically expensive and risky than sexual reproduction, and allows perennial populations to maximize clone survival and growth under favorable environmental conditions with limited disturbance (Philbrick & Les 1996). Sexual reproduction does, however, occur within these populations and seeds have facilitated perennial population recovery from mass die-off events (Plus et al. 2003; Jarvis & Moore 2010). Genetic

analyses of perennial *Z. marina* populations within Long Island, New York (USA) also suggest seedling recruitment contributed substantially to the expansion and recovery of a perennial population (Furman et al. 2015). Low levels of seedling establishment within perennial *Z. marina* populations may even occur annually, but with complete (Olesen 1999) or near total mortality, except in areas on the periphery of the denser portions of meadows or below a critical depth or disturbance threshold (Olesen 2017). These high mortality rates are attributed to competition with adult *Z. marina* shoots. These results suggest sexual reproduction is important for colonization of *Z. marina* populations, but may not be generally important for the maintenance of existing perennial meadows.

Within Chesapeake Bay *Z. marina* exhibits significant variability in growth and reproduction with the seasons and over small spatial scales (< 5 km, Johnson et al. 2017, Shields et al. 2018). Biomass of *Z. marina* peaks in the spring and early summer, declines dramatically during warm summer months, partially recovers during the fall, and once again senesces in the winter (Orth & Moore, 1986; Moore et al. 2000). Seeds are released in the spring during peak biomass (Silberhorn et al., 1983) and germinate when water temperatures drop below ~ 15 ^{o}C in the late fall and winter (Orth & Moore, 1986; Moore et al., 1993). Thus, *Z. marina* seeds in Chesapeake Bay germinate under environmental conditions favorable for growth and during a period of low adult cover and biomass. Seedlings may have an opportunity to germinate and grow within gaps between clones before peak biomass the following spring (here defined as "establishment"). This growth may establish carbohydrate reserves necessary to survive the most physiologically demanding warm summer months (here defined as "recruitment," as survival beyond these months would most likely indicate survival to reproductive age, Burke et al. 1996).

The goals of this study were to determine if *Z. marina* seedlings establish and recruit within existing meadows of *Z. marina* in Chesapeake Bay and if interactions between seedlings and surrounding adult shoots influences the survival of these seedlings. The objectives were: 1. To determine the degree to which seedlings establish within meadows of the York River; 2. To test whether seed availability influences the seedling establishment rate within meadows of the York River; 3. To quantify the relationship between seedling survival and surrounding adult vegetation; 4. To evaluate the relative influence of sexual reproduction on the maintenance of *Z. marina* meadows through space and time. This study did not explicitly test density-dependent effects of adult shoots and seedlings on one another. Instead this study evaluated competition as the outcome of interactions between adult shoots and seedlings that may compromise seedling survival and potentially impact seedling recruitment patterns.

Methods

Study Design

This study used a series of surveys and experiments over three years (2016-2018) to evaluate the influence of seed processes on perennial *Z. marina* meadows in Chesapeake Bay. Surveys and experiments were first used to evaluate natural levels of seedling establishment in the perennial meadows of Chesapeake Bay and the potential for seed supply to impact these levels. Simultaneously, experimental transplant gardens were constructed to directly test if the availability of seeds, and sexual reproduction, were necessary to maintain cover within plots relative to plots without seeds over time. In 2017, a separate survey and experiment explored the influence of surrounding vegetation on the survival of seedlings in meadows. A survey of tagged seedlings evaluated whether

seedling survival was related to the characteristics of neighboring adult shoots. Last, an experimental manipulation was used to compare the survival of seedlings with adult shoots surrounding them to the survival of seedlings around whom adult shoots had been experimentally removed.

Study Sites

Surveys and experiments were conducted in seagrass meadows at several locations in the York River, Virginia, Allens Island (AI, -76.422W, 37.257N), Bena (BE, -76.4462W, 37.2540N), Sandy Point (SP, -76.3986W, 37.2636N), Goodwin Neck (GN, -76.444, 37.297N), and Goodwin Island (GI, -76.4055W, 37.2241N), and a site located in a coastal lagoon on the Delmarva Peninsula, Spider Crab Bay (-75.820W, 37.337N) (Figure 1). All sites were shallow (< 1.0 m at MLW). We define meadows as *Z. marina* populations that are persistent spatially and temporally within aerial surveys of Chesapeake Bay submerged aquatic vegetation (Orth et al. 2017). York River sites were fringing, persistent meadows with similar tidal and thermal regimes while the coastal lagoon site was located on a shoal area within a larger bay that is part of a large scale seagrass restoration project.

Natural Seedling Establishment and Potential Seed Limitation

In order to quantify seedling establishment within meadows of *Z. marina* and to determine if the size of the seed bank at a given location may limit seedling establishment, in situ plots were constructed within the middle of seagrass meadows at GI, SP, AI, and BE in the York River. At each location, six 2 m^2 plots were constructed in three distinct blocks, each containing two plots, at similar depths (+/-10cm) in autumn

2015. Two thousand viable seeds, collected in spring, 2015 (according to Marion & Orth 2010), were then broadcast evenly onto the sediment surface within one, randomly selected plot in each block (three plots at each location). The remaining plot in each block did not receive additional seeds and was considered a control plot that would maintain the natural level of seedling establishment in the meadow that year. Four, 0.02 m^2 cores were taken from each 2 m^2 plot in late May 2016, 5 – 6 months after seed germination. The number of seedlings, the total shoot number, the maximum adult shoot height, and a random adult shoot height was then recorded for each core. Seedlings were identified as having a heavily rooted and a curved rhizome base (Setchell 1929, Figure 2(a)). This procedure was repeated in the fall and spring of 2016-2017 and 2017-2018 (Table S1). Because these plots were constructed within existing meadows, any seedlings identified from control plots, those not receiving 2000 supplemental seeds, were likely from seeds naturally settling in that area.

Adult Shoot Neighbors and Seedling Survival

In order to evaluate if the characteristics of surrounding adult shoots influence the survival of seedlings in *Z. marina* meadows of the lower Chesapeake Bay, seedlings were identified and followed monthly at GI, SP, AI, and BE in the York River. Seedlings were identified within a 4 m^2 plot at each location in April 2017. At this time of the year *Z. marina* seedlings have germinated, but the growth of *Z. marina* in Chesapeake Bay has not consolidated *Z. marina* meadows to the extent that seedlings are indistinguishable from adult shoots. Seedlings were identified as spatially isolated and lacking clonal integration with surrounding shoots. Once a seedling was identified, its position was recorded using a 1 m^2 North-South orientated grid quadrat gridded into 100, 100 cm^2 cells

for each 1 m^2 of the 4 m^2 plot. A stainless steel 19 mm washer was then slid down the leaves to the base of the shoot so that the shoot would grow through the center of the washer and anchor the washer in place along the rhizome (Figure 2(b)). The presence or absence of tagged seedlings was evaluated monthly from April - October 2017 by returning to the recorded position of the seedling and gently brushing away sediment until the lock washer was visible.

The density, height, and cover of adult shoots of *Z. marina* and *Ruppia maritima*, a sub canopy species co-occurring within *Z. marina* meadows, shoots were also recorded monthly in each 4 m^2 plot (Figure 2(d)). The number of *Z. marina* and/or *R. maritima* shoots within 16 haphazardly selected 0.02 m^2 areas was recorded within each plot. The length of one shoot representative of canopy height was recorded for each species per count. The density of shoots, representative canopy heights, and percent bottom cover taken for each 1 m^2 of the experimental area were then multiplied together to estimate a leaf area index (LAI) m^{-2} for each species at a given location for each month of the survey.

Seedling Competition Experiment

To directly test if adult *Z. marina* plants influence the survival of *Z. marina* seedlings within an established meadow, the survival of seedlings growing amongst adult shoots was compared to the survival of seedlings around whom adult shoots were experimentally removed. Twenty-one seedlings were identified and tagged with plastic coated wire bent around the base of the shoot in May 2017 within an 11 m^2 area at BE in the York River, VA (Figure 2(c)). The location of each seedling within a North-South orientated 1 m^2 grid split into 100 cm^2 cells was recorded. For 10 haphazardly selected

seedlings, all other shoots within 15 cm of the seedling were removed to eliminate any resource competition between seedlings and neighboring shoots. The shoots surrounding the remaining 11 identified seedlings were counted and left to grow around the seedlings. After a two-week treatment acclimation period, to account for any mortality resulting from the application of treatments, the presence and survival of each tagged seedling was recorded weekly through October 2017.

Transplant Garden Experiments

To test if the establishment of seed banks by sexual reproduction within existing meadows of *Z. marina* is important to maintain the bottom cover of mature *Z. marina* meadows, an experimental manipulation of adult *Z. marina* plants and seed banks was initiated in fall 2015. Thirty-two experimental *Z. marina* plots were constructed in bare sediment in eight rows of four, $1m^2$ plots at Goodwin Neck, just upstream of a persistent *Z. marina* meadow. One plot in each row was planted with: 1. a known density of adult *Z. marina* plants ($70\ m^{-2}$) and *Z. marina* seeds ($1000\ m^{-2}$); 2. a known number of adult *Z. marina* plants ($70\ m^{-2}$); 3. a known number of *Z. marina* seeds ($1000\ m^{-2}$); and 4. neither seeds nor adult *Z. marina* transplants. Each of the eight rows contained one plot of each treatment. The percent of *Z. marina* bottom cover was then evaluated monthly in each plot from May to October over three years, 2016-2018, to determine if the presence of a seed bank was crucial for the long-term persistence of the plots. Seedlings of *Z. marina* and *R. maritima* detected from aerial photography in 2015 suggested this experimental area would be suitable for *Z. marina* growth. All flowering shoots were removed from plots to ensure the only sexual reproduction within plots stemmed from experimental treatments.

In order to scale up the transplant garden experiment spatially and test if the benefits of sexual reproduction vary with location, the experimental design described above was replicated with larger plots at two locations. Sixteen 4 m^2 plots were constructed in four rows of four plots in the York River, ~ 35m from the 1 m^2 plots mentioned above, and in Spider Crab Bay. One replicate of each treatment described above was haphazardly placed in each of the four rows. The density of transplants was lowered to 70 transplants per plot (4 m^2) in order to simulate published restoration techniques (Orth et al. 1999). 1000 seeds m^{-2} were added to all seeded plots. Plots were constructed in fall 2016 and the bottom cover (m^{-2}) of each plot was evaluated monthly from May to October in 2017 and 2018.

Statistical Analyses

Natural Seedling Establishment and Potential Seed Limitation

A generalized linear model (GLM) fit to a Poisson distribution was used to determine if the number of seedlings naturally establishing in meadows of the York River varied by location and/or year (Table S2). A generalized linear mixed effects model, also fit to a Poisson distribution, was then used to evaluate if the seed addition treatment significantly increased the number of seedlings within cores taken from plots with additional seeds relative to control plots at a given location in a given year. For this model the block, location, and year of sampling were treated as nested random variables. A mixed-effects model was used to test if mean seedling heights were significantly shorter than the mean height of surrounding, adult shoots within cores. The year and location from which shoot heights were measured were considered random variables. The heights of shoots were $\log_{10}$ transformed to meet model assumptions.

Adult Shoot Neighbors and Seedling Survival

A first order auto-regressive model fit to a Gaussian distribution was used to test if the mean numbers of seedlings surviving in an area was related to the mean estimated LAI of *Z. marina* or *R. maritima* shoots surrounding the seedling. The estimated LAI of *Z. marina* and *R. maritima* were considered fixed variables, but the month and the location at which seedlings were evaluated were considered nested, random variables. Due to the radically different scales between the LAI of *Z. marina* and *R. maritima*, these variables were scaled with the *scale* function in the base R package. Because bottom cover was not recorded at Goodwin Island in July, an estimate of bottom cover was made by averaging bottom cover taken in June and August.

Seedling Competition Experiments

Kaplan-Meier survival curves were generated for seedlings growing with and without neighboring, adult *Z. marina* shoots. A log-rank test was then used to compare these survival curves to determine if the duration of seedling survival differed between these two treatments.

Transplant Garden Experiments

Differences in percent bottom cover between plots with eelgrass seeds, eelgrass plants, and both eelgrass plants and seeds were determined with first-order auto-regressive models. The eelgrass plant treatment was considered the referenced control for all comparisons to determine if treatments with sexual reproduction differed in bottom cover from plots with only asexual reproduction. Bottom cover estimates were square root transformed to meet model assumptions. Analysis of deviance was then used to

compare model terms. For the 1 m^2 experiments, the reproductive treatment was treated as a fixed effect while the year, month, and row in which cover was evaluated were treated as nested, random effects. For the 4 m^2 experiments, the reproductive treatment and location of the experiment were treated as interacting fixed effects while the year, month, row, and plot in which cover was evaluated were treated as nested, random effects. Because all estimates of bottom cover during the first year may represent transplantation success more than functional survival through time, additional first-order auto-regressive models were also constructed to evaluate the impact of the treatments on percent bottom cover after the first year of growth for both experiments. Bare sediment control plots were not included in these comparisons, as these plots were used to evaluate background seedling establishment rather than for any comparison of long-term bottom cover.

All statistical analyses were performed in R statistical analysis software (R Development Core Team, 2018). A type I error rate of 0.05 was established for all statistical tests. Coefficients of generalized linear models are reported as odds ratios derived from the back transformed model coefficient. Generalized linear and mixed effect models were built with the *glm* and *glmer* functions in the *lme4* package (Bates et al. 2015). Linear mixed effect and autoregressive models were constructed using the *lme* function from the *nlme* packages (Pinheiro et al. 2018). Kaplan Meier curves and log-rank tests were conducted with the *survfit* and *survdiff* functions from the *survival* R package (Therneau & Lumley 2018). Model assumptions were assessed graphically and estimates of dispersion in GLMs were calculated manually or with the *dispersion_glmer* function in the *blmeco* package (Korner-Nievergelt et al. 2015).

Results

Natural Seedling Establishment and Potential Seed Limitation

Naturally established seedlings were identified in control plots at all locations over the course of the three study years (Figure 3). On average, more seedlings were found at GI (126 ± 65 seedlings m^{-2}) than at AI (55 ± 14), SP (50 ± 16), or BE (24 ± 7). More seedlings were also found in 2018 (125 ± 49 seedlings m^{-2}) than in 2016 (45 ± 13) or 2017 (21 ± 5). The effect of location on seedling establishment varied significantly with the year of sampling (Table S3, $p < 0.001$). All locations except GI demonstrated higher seedling establishment in 2018 relative to 2016 and 2017. Goodwin Island exhibited high seedling establishment in both 2016 and 2018. The significant interaction between the location and the year of sampling on seedling establishment likely stems from this high seedling establishment at GI in both 2016 and 2018. Seedlings also made up the largest fraction of the total shoots present at GI (19 ± 30%) relative to SP (16 ± 26%), AI (10 ± 20%), or BE (3 ± 6%). Mean seedling heights (17 ± 0.46 cm) were consistently shorter (Table S4, $\beta = 0.42 \pm 1.1$, $t = -6.1$, $p < 0.001$) than the mean height of surrounding vegetation (44 ± 1.7cm) across locations. The average difference in height between seedlings and surrounding vegetation was smaller at GI (8.5 ± 3.6 cm difference, Figure S1) than at other locations (SP: 27 ± 11 cm, BE: 30 ± 5.8 cm, AI: 40 ± 3.4 cm). A significant increase in the number of established seedlings was detected between plots with an additional 1000 seeds m^{-2} (Table S5, $\beta = 2.0 \pm 1.1$, $z = 6.7$, $p < 0.001$) relative to control plots over the three study years and across all four locations.

Adult Shoot Neighbors and Seedling Survival

The mean number of seedlings present m^{-2} was negatively related to the calculated LAI of *Z. marina* (Table S6, $\beta = -0.09 \pm 0.03$, $t = -3.5$, $p < 0.001$) but positively related to the calculated LAI of *R. maritima* ($\beta = 0.09 \pm 0.02$, $t = 3.6$, $p < 0.001$). The measured leaf area index (LAI) for *Z. marina* was higher at BE (3245 ± 212.8, Figure S2) than AI (1987 ± 124.8), GI (651.6 ± 23.25), or SP (133.5 ± 17.10). The LAI of *R. maritima* was generally lower than the LAI for *Z. marina* and was higher at GI (430.0 ± 25) than SP (1.2 ± 0.5), BE (0.26 ± 0.13), or AI (0.068 ± 0.0039). The vast majority of seedlings disappeared between June and September 2017 (90 %). Only seedlings at Goodwin Island (n = 6) and Bena (n = 1) survived into the fall growth period for *Z. marina* in Chesapeake Bay (Figure 4). Extensive algal mats appeared at SP in June 2017. All *Z. marina* and *R. maritima* within the SP plot were gone by the end of the summer (Figure S3). These mats did not occur at other study locations.

Seedling Competition Experiments

The survival of seedlings without neighboring shoots was significantly higher than the survival of seedlings with neighboring shoots ($\chi^2 = 12.4$, $p < 0.001$). In fact, the only seedlings to survive the experimental period were seedlings without neighboring shoots within a 15 cm radius (Figures 2(c) & 5).

Transplant Garden Experiments

Percent bottom cover changed seasonally within all 1 m^2 reproductive treatment plots in the York River (Figure 6). The highest mean bottom covers generally occurred around June and the lowest mean cover in September and October. The percent bottom cover of plots with sexual reproduction (the transplanted and seeded or solely seeded

treatments) did not significantly differ from plots with exclusively asexual reproduction (the solely transplanted treatment) across all three years of the study ($F_{2,286}$ = 0.68, p = 0.5). Bottom cover within seeded plots did, however, differ significantly ($F_{2,190}$ = 10.6, p < 0.001) from transplant plots after 2016 (i.e. in 2017 and 2018). By October 2018, the bottom cover of all plots in the York River had declined sharply.

The larger, 4 m^2 reproductive treatment experiment demonstrated strong differences between locations. The effect of the seeded treatment on bottom cover interacted with the locations of the study (Table S7, $F_{2,1005}$ = 13.3, p < 0.001). The experimental plots in the York River exhibited similar seasonal trends in percent bottom cover to the adjacent 1 m^2 plots, but were less stable and higher in peak bottom cover than plots within Spider Crab Bay (Figure 7). By October 2018, however, bottom cover within the York River declined dramatically relative to those in Spider Crab Bay. The seeded treatments, in particular, maintained higher bottom cover within the York River than the equivalent treatments in Spider Crab Bay. Interestingly, plots with adult plants (i.e. the transplanted and seeded or solely transplanted treatments) always maintained the highest cover in Spider Crab Bay.

Discussion

Our results demonstrate seedling establishment consistently occurs within the perennial *Z. marina* meadows of the lower Chesapeake Bay, but the magnitude of establishment varies substantially over space and time. Seed availability or the size of the seed bank may partially explain the observed variability in the seedling establishment rate. Unexplored factors, such as local seed predation (Fishman & Orth 1996) or winter

storms (Marion & Orth 2012, Jarvis & Moore 2015), can also, however, markedly influence seedling establishment rates. Established seedlings contributed to the structure of meadows during peak biomass in this region (as high as 20% of shoots). A small proportion of established seedlings also survived to recruit into the reproductive population within surveyed meadows. Interactions between seedlings and surrounding vegetation may be one of several critical factors influencing the survival of seedlings within existing meadows. Seedling recruitment, and therefore sexual reproduction, appear to be more important in meadow maintenance at locations with regular disturbances that create patches of bare sediment within the meadow. Disturbances may potentially generate gaps in the existing population that open opportunities for greater survival of seedlings within the meadow. Sexual reproduction may thus play an irregular but important role in the maintenance of perennial *Z. marina* meadows.

Role of sexual reproduction: disturbance-driven relevance

As a species, *Zostera marina* invests variably in and relies variably on sexual reproduction for population survival. The relevance of sexual reproduction to population survival occurs along a continuum, from essential for annual populations, entirely dependent on seedling recruitment, to unnecessary, for perennial populations with little or no flowering. Results in this study support these general findings with surveyed perennial populations in the lower Chesapeake Bay recording similar or slightly higher levels of seedling establishment to perennial populations in China (Xu et al. 2018) and the Baltic Sea (Olesen 2017). Similarly, few seedlings within the perennial meadows of Chesapeake Bay survived to recruit into the population as in other locations. The observed variability

across populations of perennial *Z. marina* meadows recorded in this study, however, suggests the importance of sexual reproduction for population maintenance may change over small spatial and temporal scales. For example, higher levels of establishment and recruitment at Goodwin Island relative to other locations demonstrates perennial populations may rely differently on sexual reproduction over small spatial scales ($\leq$ 5 km). Similarly, higher levels of seedling establishment in 2018 relative to 2016 and 2017 suggests the importance of sexual reproduction for perennial populations in Chesapeake Bay may shift between years. Results from transplant experiments suggest the role of sexual processes in perennial meadow maintenance may depend on the extent to which disturbance, associated with summer water temperatures in Chesapeake Bay, damages the existing meadow. Within the York River, plots receiving seeds performed better than plots without seeds only after years with substantial declines in overall cover (i.e. cover was higher in seeded plots than just transplant plots in 2017 after declines in 2016). These results support models hypothesizing the pivotal role of sexual reproduction for population recovery after extreme die-off events (Jarvis et al. 2014), but also potentially for augmenting the recovery from seasonal die-backs of varying severity. Although severe disturbances may themselves compromise seedling survival, disturbance may also potentially lower intraspecific competition precluding seedling recruitment among adult shoots and enhance seedling survival (Yang et al. 2016). The overall reproductive plasticity of *Z. marina* confirms a general pattern of recruitment for the species does not exist, but that individual populations likely adapt reproductive patterns, whether ISR or RSR, capable of maintaining and restoring the population in the face of the disturbance.

Observations of seedling establishment and the increased importance of sexual reproduction at locations and in years with low adult cover suggest a reproductive bet hedging strategy within the perennial *Z. marina* meadows of Chesapeake Bay. During years of high disturbance, e.g. years with high summer water temperatures and/or poor water quality, low numbers of adult shoots will survive, and seedlings germinating after environmental conditions improve will likely constitute more of the shoots the following spring. During years with less seasonal disturbance, e.g. years with cooler summer water temperatures and/or better water quality, more adult shoots will survive through the year and the relative influence of seedlings will likely be low compared to the growth of these overwintering shoots the following spring. During these years, shoots of existing plants likely outcompete seedlings germinating within the meadow. The relative role of sexual reproduction in meadow maintenance would thus change along a gradient of disturbance to the existing canopy so long as seed production and environmental conditions suitable for seedling establishment are maintained.

Such selection for reproductive strategies to maintain populations likely exists more broadly. Sexual reproduction may serve an important role in *Z. marina* population survival at locations with discrete seasonal or cyclical disturbances, such as ice scour (Robertson & Mann 1984), seasonal light disruptions (Kim et al. 2014), or high temperatures (Santamaria-Gallegos 2000; Jarvis et al. 2012; this study) to the existing population, but which subside or disappear by the time seeds germinate. Conversely, at locations without acute seasonal disturbances or a strong potential for disturbance at some point during seedling establishment, asexual reproduction may play a larger role in maintaining individuals and populations (Reusch et al. 1999, Billingham et al 2003). The

variability and importance of sexual reproduction in this study and across the distribution of *Z. marina* suggests the plasticity of reproductive traits among *Z. marina* populations may facilitate the species' colonization of diverse environments and habitats.

Potential Impact of Seed Supply and Establishment

For sexual processes to accelerate meadow recovery from seasonal disturbances, seed production and banks must be sufficient at locations where a disturbance occurs and the disturbance itself cannot also disturb the seed or seedling bank. Flowering intensity and seed bank densities fluctuate in both space and time (Silberhorn et al. 1983, Harwell & Orth 2002). Results from seed addition experiments in this study suggest seed availability or supply may influence the number of established seedlings in Chesapeake Bay. As a result, seedling recruitment may not reliably fill gaps that disturbances open in seagrasses canopies in Chesapeake Bay. In addition, for perennial populations within the lower Chesapeake Bay, seedlings will generally flower for the first time in their second year of growth (Orth & Moore 1983; Jarvis & Moore 2010). Multiple acute disturbances, e.g. consecutive years of highly stressful summers, may thus dramatically reduce the flowering population and seed supply. With simultaneous low shoot survival and seed supply, seagrass populations will struggle to recover (Jarvis & Moore 2010; Jarvis et al. 2014). As a result, the reproductive plasticity of a perennial population may have a limited capacity to aid population recovery.

The intensity and timing of disturbances may also alter the relative benefit of sexual or asexual reproduction. Unlike in the York River, bottom cover within the seeded plots in Spider Crab Bay transplant garden experiments was consistently lower than in

plots with adult transplants. The lower cover in seeded plots in Spider Crab Bay likely results from consistently higher wave and tidal current energy at this location relative to the York River during seedling establishment (Figure S4*)*. Colder and clearer water within coastal lagoons of the Delmarva Peninsula may also favor shoot survival and asexual reproduction in Spider Crab Bay relative to the York River location (Moore et al. 2012). The combined impact of higher energy during vulnerable seedling establishment periods and high water quality may select for higher clonal than seedling survival in Spider Crab Bay. Of course, disturbances during seed settlement or seedling establishment, even if away from adult vegetation, may also diminish the role of sexual reproduction in meadow maintenance (Marion & Orth 2012; Yang et al. 2016). Should disturbances increase in frequency and intensity before or during seed production or seedling establishment, the resilience of populations reliant on sexual reproduction may decline and population collapse may occur.

Impact of Adult Shoots on Seedlings

Although previously hypothesized (Olesen 1999, 2017), this study is the first to observationally and experimentally demonstrate a relationship between surrounding vegetation and seedling survival within existing meadows. Adult shoots could compete with each other and seedlings for common resources, such as space, light, or nutrients (Williams 1987; Gopal & Goel 1993, Invers et al. 2001, Gustafsson & Boström 2016). Interestingly, the location with the lowest measured leaf area index, Sandy Point, was smothered in extensive and unexpected algal mats during the summer of 2017 (Figure

S3). For this location, interactions with macroalgae, not surrounding seagrass shoots, likely compromised seedling survival. Although resource competition, whether inter or intraspecific, may not immediately or directly threaten seedling survival, the lower resource levels left for seedlings after adults draw down common resource pools could negatively impact seedling growth (Bintz & Nixon 2001, Zhang et al. 2014, Figure S5 & S6). For *Z. marina* in Chesapeake Bay near the southern limit of its distribution, growth during optimal environmental conditions is important for accumulating non-structural carbohydrate reserves needed to survive during stressful environmental conditions (Burke et al. 1996). Because seeds germinate in early winter in Chesapeake Bay and have the potential to grow in gaps before interacting with adult shoots in the spring, the timing and growth of seedlings prior to experiencing stressful summer environmental conditions could be critical to their survival and should be investigated more thoroughly (Orth & Moore 1983, Figures S7 & S8). By slowing growth and the accumulation of reserves, resource competition could dramatically hamper seedling survival.

Some seedlings did, however, survive the most stressful summer period at two of the locations studied. This survival suggests competition among adult clones does not entirely preclude seedling recruitment in perennial populations of *Z. marina*. The majority of the surviving seedlings were growing near Goodwin Island. This location maintained the second lowest estimated LAI of *Z. marina*, suggesting intraspecific competition at this location may have been lower than at other locations. In addition, seedlings at Goodwin Island were much closer to canopy height than at all other locations. The lower LAI and smaller difference in height between seedlings and adults

of *Z. marina* at Goodwin Island may not have generated strong intraspecific competition for resources on vulnerable seedlings as at other studied locations.

Conclusions

The relative role of sexual reproduction in *Z. marina* meadow maintenance is likely a function of both seed supply and the survival of propagules competing with surrounding clones. Seedling establishment is an important demographic process within the perennial *Z. marina* meadows of Chesapeake Bay but varies across locations. Seed availability may partially explain this variability in establishment among locations. Interactions between surrounding shoots and established seedlings appears to limit the survival of seedlings within existing meadows through the stressful summer period in Chesapeake Bay. Sexual processes will likely be important for *Z. marina* population dynamics where seed production is high and clonal survival is low. Biotic (e.g. ray or crab holes, macroalgal mats) or abiotic (e.g. stressful water temperatures or strong wave energy) disturbances lead to low clonal survival. These disturbances to the population may provide windows of opportunity for seedlings to germinate and survive, so long as they are offset in time from seed germination and development.

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Figures

Figure 1:

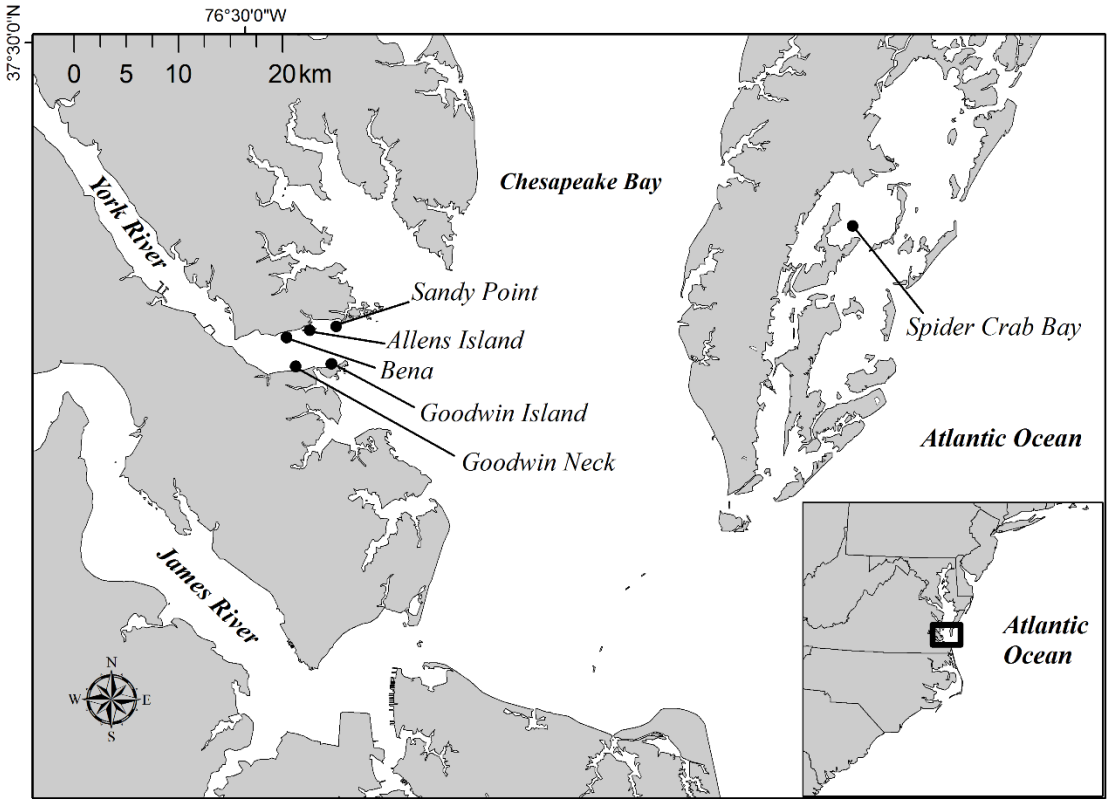

Figure 1. The location of experiments and surveys throughout the lower Chesapeake Bay and Eastern Shore of Virginia.

Figure 2:

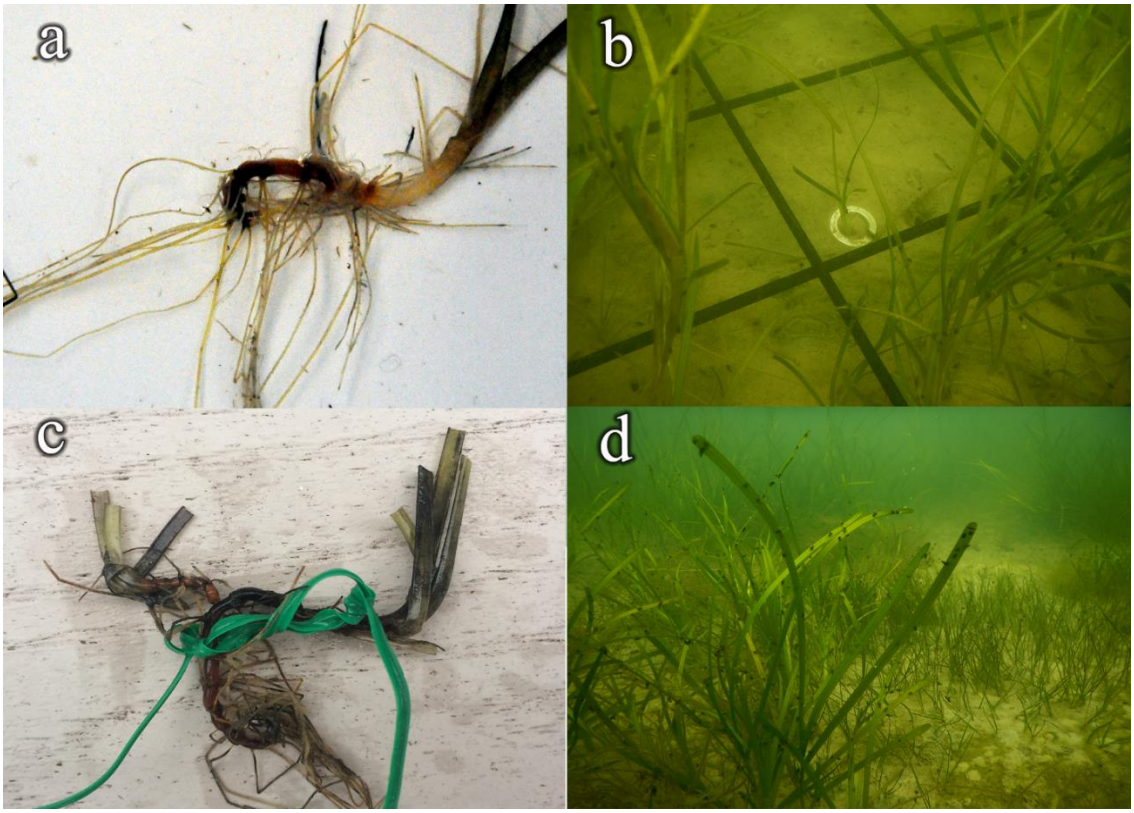

Figure 2. Seedlings and adult shoots of *Z. marina*: (a) the distinctive hook and "hairy" end to the rhizome used to identify seedlings in the study, (b) a seedling identified *in situ* with a 19 mm lock washer about to be placed around its rhizome (c) a dead seedling dug up from the competition treatment after defoliating (d) a mixed *Z. marina* (longer leaves, foreground) and *R. maritima* (shorter leaves, middle ground) meadow at Goodwin Island

Figure 3:

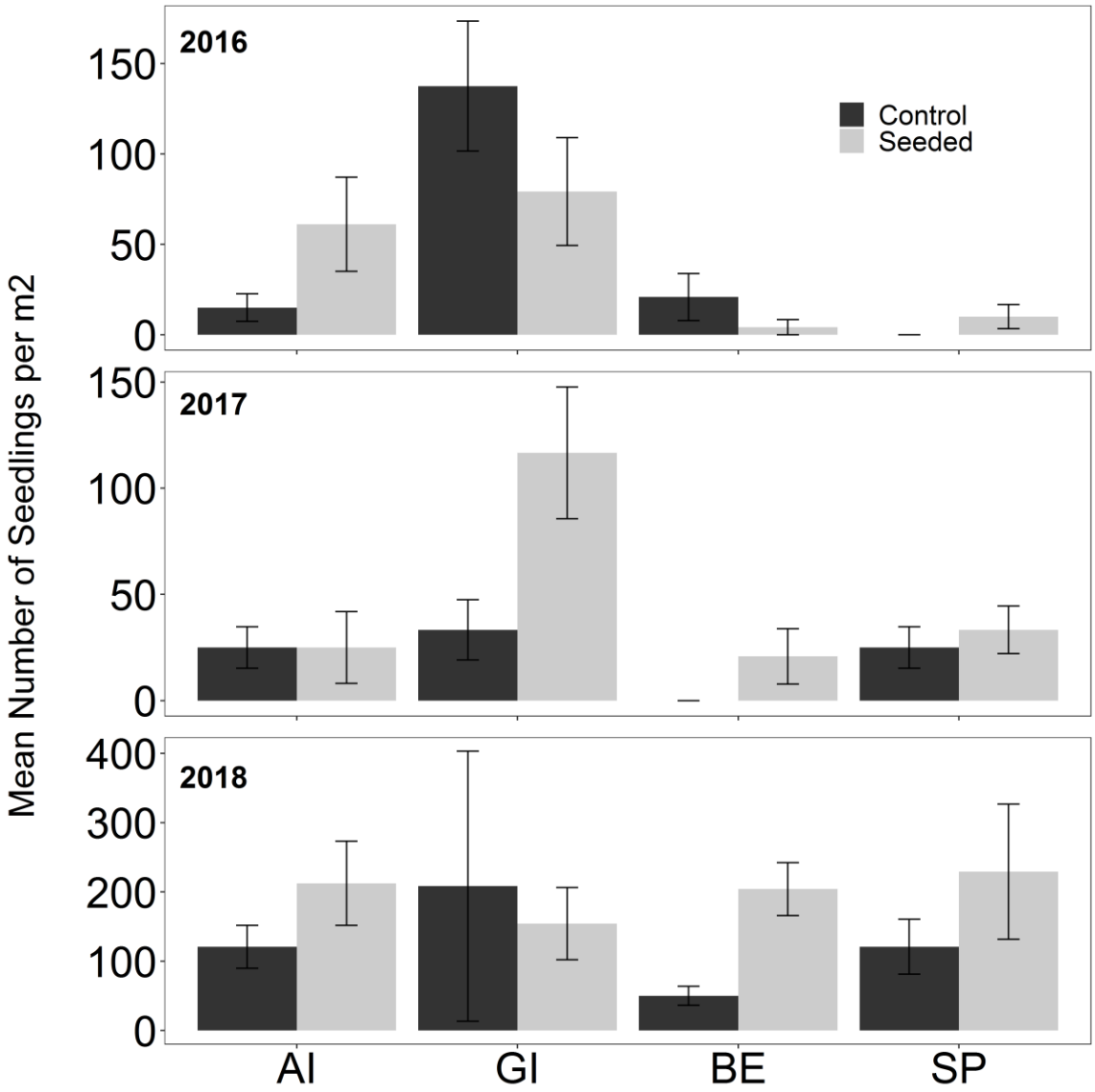

Figure 3. The mean (± SE) number of seedlings found in 0.02 m^2 cores (n = 12) taken from plots at sample locations in the York River from 2016 – 2018.

Figure 4:

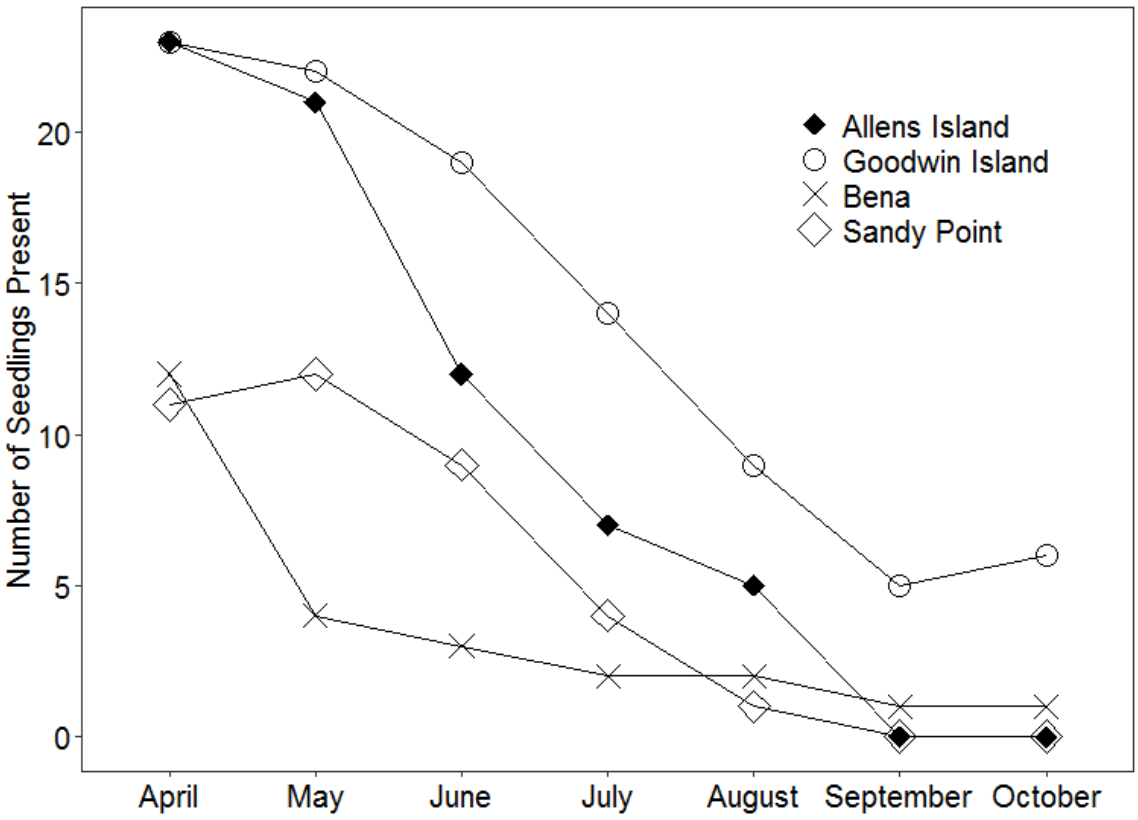

Figure 4. The number of tagged seedlings found during monthly sampling at Allens Island (n = 24), Goodwin Island (n = 23), Bena (n = 12), and Sandy Point (n = 11). While sampling in May 2017, an additional seedling was identified and tagged at Sandy Point. Similarly, a tagged seedling that was not found in September, was discovered alive in October at Goodwin Island.

Figure 5:

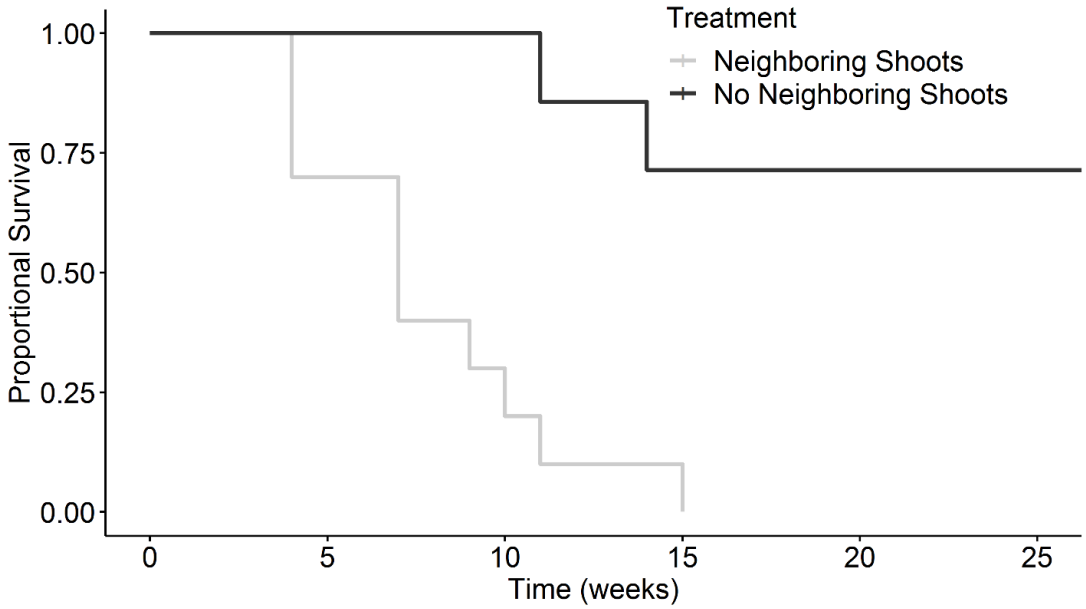

Figure 5. The proportional survival of seedlings with (grey, n = 10) and without (black, n = 7) neighboring shoots.

Figure 6:

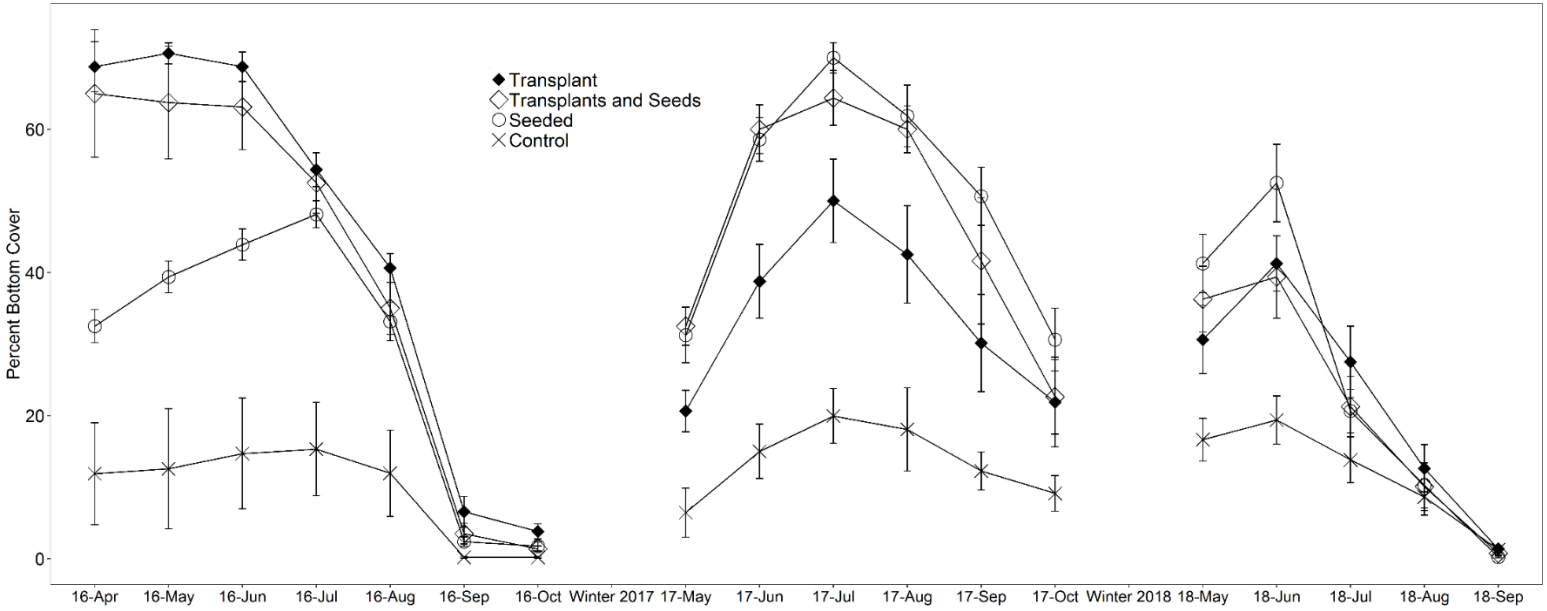

Figure 6. The mean (± SE) percent bottom cover from 2016-2018 of 1 m^2 experimental plots (n = 8 per treatment) built within unvegetated sediment of the York River, VA in fall 2015.

Figure 7:

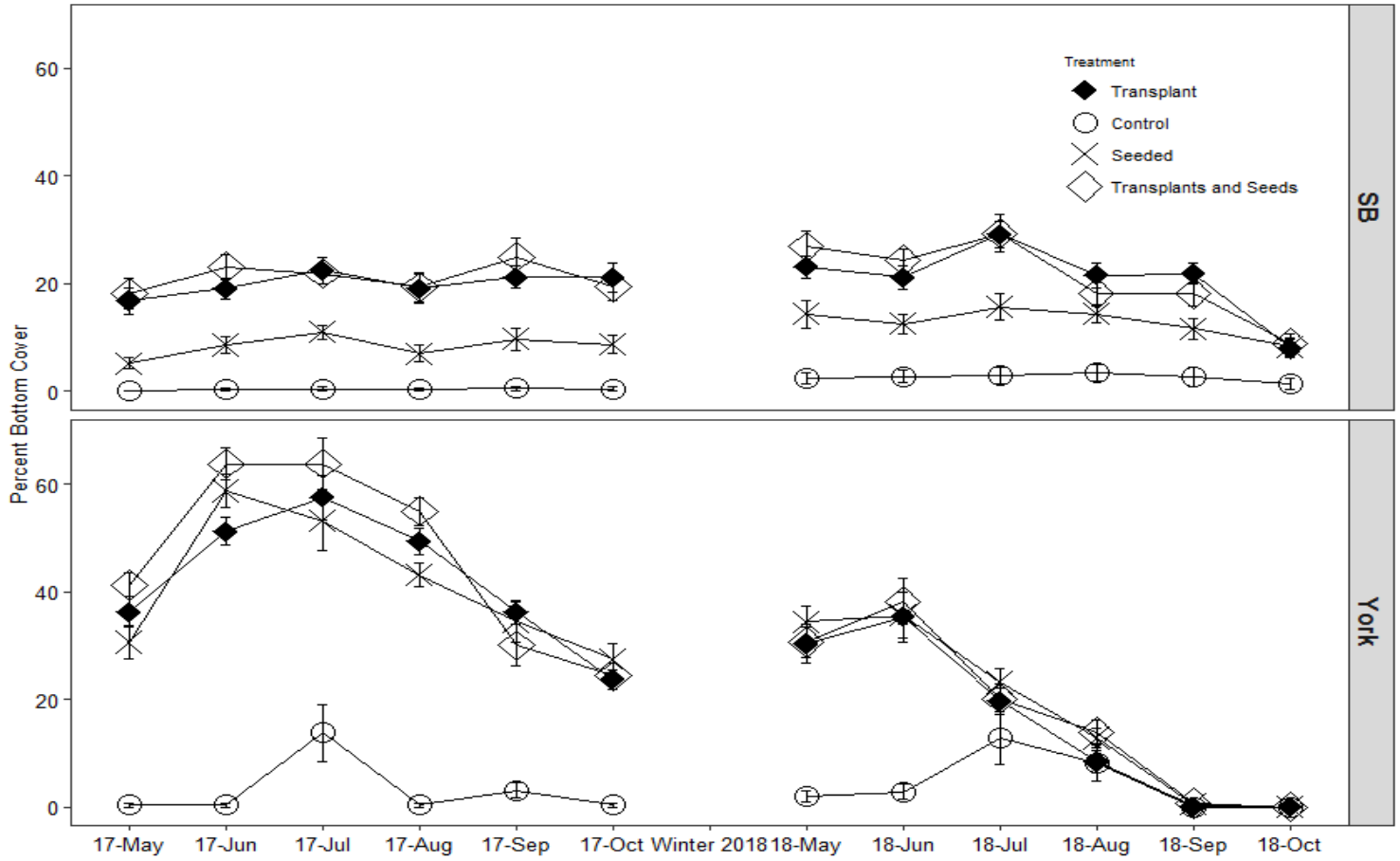

Figure 7. The mean (± SE) percent bottom cover from 2017 and 2018 in 4 m^2 experimental plots (n = 4 per treatment) built within unvegetated sediment of the York River, VA (York) and Spider Crab Bay, VA (SB) in fall 2016.

SYNTHESIS

The chapters of my dissertation explored: 1. The impact of disturbance from sediment bioturbators on seedling recruitment for the marine seagrass *Posidonia australis* in Western Australia; 2. The role of herbivory, likely from a common, estuarine, omnivore, *Callinectes sapidus*, on seedling recruitment of a freshwater/oligohaline SAV species, *Vallisneria americana*, within an oligohaline region of the Chesapeake Bay; 3. The prevalence of seedling establishment and the potential for intraspecific competition to influence seedling survival and recruitment for the seagrass *Zostera marina* in the estuarine Chesapeake Bay. In aggregate these three chapters broadly demonstrated that three different biological interactions, disturbance, herbivory, and competition, in three different coastal environments, a coastal lagoon, an estuary, and an oligohaline system, can substantially impact seedling survival. This research complements and broadens previous research in terrestrial (Eriksson 1989, Leck et al. 2008, Mole & Westoby 2004) and aquatic (Dumbauld & Wyllie-Echeverria 2003, Valdermarsen et al. 2011, Fishman & Orth 1996) environments demonstrating that biota can impact the early life stages of plants and thereby alter the subsequent plant community.

The three biological interactions investigated in this dissertation represent direct and indirect biological interactions that have acute and chronic impacts on seedling survival. Chapter One shows that three sediment detritivores, and likely a diverse array of other sediment bioturbators that dig, plow, or excavate the sediment, can limit seedling recruitment in locations where their populations overlap with suitable SAV habitat (Dumbauld & Wyllie-Echeverria 2003, Valdermarsen et al. 2011). Bioturbator foraging that incidentally and repeatedly dislodges *P. australis* seeds could prevent seeds from ever becoming incorporated into the sediment or expose them to seed predators. These

unintentional disturbances have a direct and immediate impact on seedling state in the sediment. The dislodging of seeds can ultimately have an acute (from seed predators) or chronic (by preventing sufficient root anchors) impact on seed survival. Sediment bioturbators are found worldwide in benthic environments (Kristensen et al. 2012) that support seagrasses, thus, their influence on seagrass persistence may be severely underestimated. Chapter Two demonstrates that even grazing on isolated seedlings and propagules can greatly inhibit sexual recruitment of *V. americana* into otherwise adequate SAV habitat. In this chapter, seedling and propagule survival was observed to be compromised from repeated clipping, likely by *C. sapidus*. This targeted clipping has an immediate impact on seedlings and propagules, as herbivores remove leaves from plants. Plants may survive this initial grazing event, but repeated clipping likely prevents *V. americana* seedlings and propagules from sustaining sufficient photosynthetic area and metabolic resources to survive. Other SAV species in the system with higher potential growth and propagule production appear to be able to overcome this degree of herbivory. While many grazing studies on SAV have focused on mega-consumers such as dugongs, manatees, turtles, and waterfowl (Fourqurean et al .2010, Bakker et al. 2016) directly targeting stands of vegetation, this study suggests that an opportunistic marine invertebrate can alter SAV population recovery. Finally, Chapter Three investigates the consequences of chronic, indirect, and intraspecific interactions, most likely competition for limiting resources, on the long-term survival of seedlings growing among adult shoots. Established adult shoots may more efficiently and effectively exploit resources than seedlings around them (Zimmerman 2003). Competition for resources between adult shoots and seedlings may slow the accumulation of resources during favorable growth

conditions needed to survive during more stressful conditions (Burke et al. 1996). This resource drain, in combination with other metabolic stresses, could result in a prolonged death spiral for seedlings growing among adult shoots. Combined these three chapters and mechanisms of seedling mortality demonstrate the variability and strength through which biotic interactions can influence seedling survival and recruitment across a broad range of SAV communities and coastal environments. By impacting the success of sexual reproduction, these interactions could generate bottlenecks to SAV population growth, recovery, and resilience across species assemblages and environments.

In all three chapters, the described biological interactions will combine with other biotic and/or abiotic forces also present in each system to further increase the probability of seedling mortality. For *P. australis* seeds and seedlings growing in energetic, coastal systems, the presence of strong wave energy will also disturb seeds (Statton et al. 2017). In calmer environments, seed predators may discover seeds that bioturbators push out of the sediment (Orth et al 2002, 2006, 2007). Similarly, *C. sapidus* or other herbivores that rapidly and consistently clip *V. americana* propagules may reduce the ability of a plant to capture light. In combination with the added stress of a marginal light environment for growth found in this higher turbidity region, consistent clipping reduces plant photosynthesis and ultimately reduces the metabolic resources of a seedling or propagule (Carter & Rybicki 1985). Lastly, *Z. marina* seedlings growing in and amongst shoots of established *Z. marina* likely cannot compete as efficiently for limiting resources like space or light as adult plants (Bintz & Nixon 2001, Zimmerman 2003, Zhang et al. 2014). Such resource competition, if not immediately or directly compromising survival, lowers

the availability of critical resources necessary to survive during stressful environmental periods later (Burke et al. 1996, Yang et al 2018).

Combined abiotic and biotic forces will generate a "sieve" to seedling recruitment as defined by Harper (1977). The biological and abiotic forces and their interactions constitute the "threads" of this sieve that will likely fluctuate in intensity with space and time as biotic and abiotic interactions change. To most effectively determine the potential bottlenecks to seedling recruitment in the future, the impacts of both abiotic and biotic forces on seedling survival should be considered in future investigations, not only individually, but in tandem and interactively.

By further quantifying the biological factors limiting seedling recruitment, this dissertation will not only improve the ability of resource managers and scientists to better understand, model, and predict SAV population dynamics, but also to optimize seed based mitigation and restoration strategies in coastal environments. For example, understanding the potential for disturbance to impact interactions between adult shoots and seedlings informs our predictions for population stability and recovery by more accurately defining the role of sexual reproduction in the annual dynamics of SAV populations. Similarly, recognition of the impact of biota on potential seedling recruitment in a restoration area may increase the odds of restoration success, with measures compensating for higher early life-stage mortality or strategies to bypass a discerned bottleneck to recruitment. In general, the studies in this dissertation demonstrate the potential for biotic agents to limit seedling recruitment success, reduce natural SAV expansion and resilience, and inhibit seed-based restoration efforts. Additionally, the diverse environments across which this work was conducted suggest the

widespread potential for biological interactions to act in combination with each other and/or abiotic stressors to strongly impact seedling recruitment. These findings suggest that in order to accurately predict the population dynamics of SAV species and to optimize their restoration, both the abiotic and biotic limitations to sexual reproduction, often the most vulnerable life stages in the life history of a plant, must be more fully considered.

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CHAPTER ONE SUPPLEMENTARY MATERIAL

Supplementary Tables

Table S1

Table S1. Tukey HSD multiple comparisons of means (difference in means) between the number of sand dollars counted along transects at locations around Cockburn Sound (CS), three sites in Owen Anchorage (OAN, OAC, OAS), and Carnac Island (CI). 95% family-wise confidence levels are displayed below. Counts were $1og_{10}$ transformed to meet model assumptions.

Location	CS	OAS	OAC	OAN	CI
CS	1				
OAS	-0.89**	1			
OAC	-0.03	0.86**	1		
OAN	-0.59**	0.3	-0.55**	1	
CI	-0.95**	-0.06	-0.92**	-0.36*	1

*** = p < 0.05**

**** = p < 0.001**

Table S2

Table S2. Tukey HSD multiple comparisons of means (difference in means) between the number of sea stars counted along transects at locations around Cockburn Sound (CS), three sites in Owen Anchorage (OAN, OAC, OAS), and Carnac Island (CI). 95% family-wise confidence levels are displayed below. Counts were $1og_{10}$ transformed to meet model assumptions.

Location	CS	OAS	OAC	OAN	CI
CS	1				
OAS	0.56*	1			
OAC	0.62*	0.06	1		
OAN	-0.19	-0.75*	-0.81**	1	
CI	-0.37	-0.93**	-0.99**	-0.18	1

*** = p < 0.05**

**** = p < 0.001**

Table S3

Table S3. Tukey HSD multiple comparisons of means (difference in means) between the number of seeds counted along transects at locations around Cockburn Sound (CS), three sites in Owen Anchorage (OAN, OAC, OAS), and Carnac Island (CI). 95% family-wise confidence levels are displayed below. Counts were $1og_{10}$ transformed to meet model assumptions.

Location	CS	OAS	OAC	OAN	CI
CS	1				
OAS	1.8**	1			
OAC	0.65**	-1.2**	1		
OAN	1.0**	-0.80**	0.39*	1	
CI	2.0**	0.10	1.3**	0.90**	1

*** = p < 0.05**

**** = p < 0.001**

Table S4

Table S4. Tukey HSD multiple comparisons of means (difference in means) between the number of seedlings counted along transects at locations around Cockburn Sound (CS), three sites in Owen Anchorage (OAN, OAC, OAS), and Carnac Island (CI). 95% family-wise confidence levels are displayed below. Counts were $1og_{10}$ transformed to meet model assumptions.

Location	CS	OAS	OAC	OAN	CI
CS	1				
OAS	0.45	1			
OAC	0.88**	0.43	1		
OAN	0.20	-0.25	-0.68*	1	
CI	0.38	-0.074	-0.50*	0.18	1

*** = p < 0.05**

**** = p < 0.001**

Supplementary Figures

Figure S1

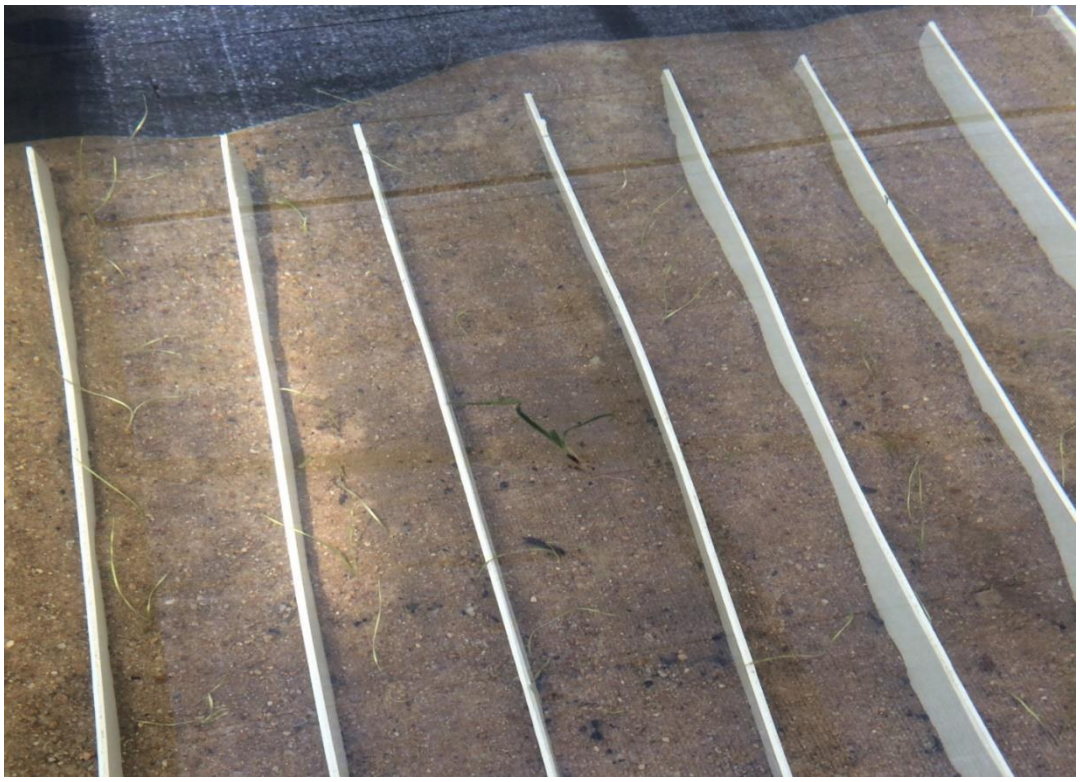

Figure S1. A floating *P. australis* seed less than two weeks after release from its fruit.

Figure S2

https://drive.google.com/file/d/1_DSHl_TzUCPfoxajSyCNRR81DDNxJrMu/view?usp=sharing

Figure S2. Recordings of preliminary experiments guiding bioturbators into seeds (green and oval shaped), on the surface and buried 1 cm into the sediment, and one-year-old seedlings. Please disregard dates and names on title slides.

Figure S3

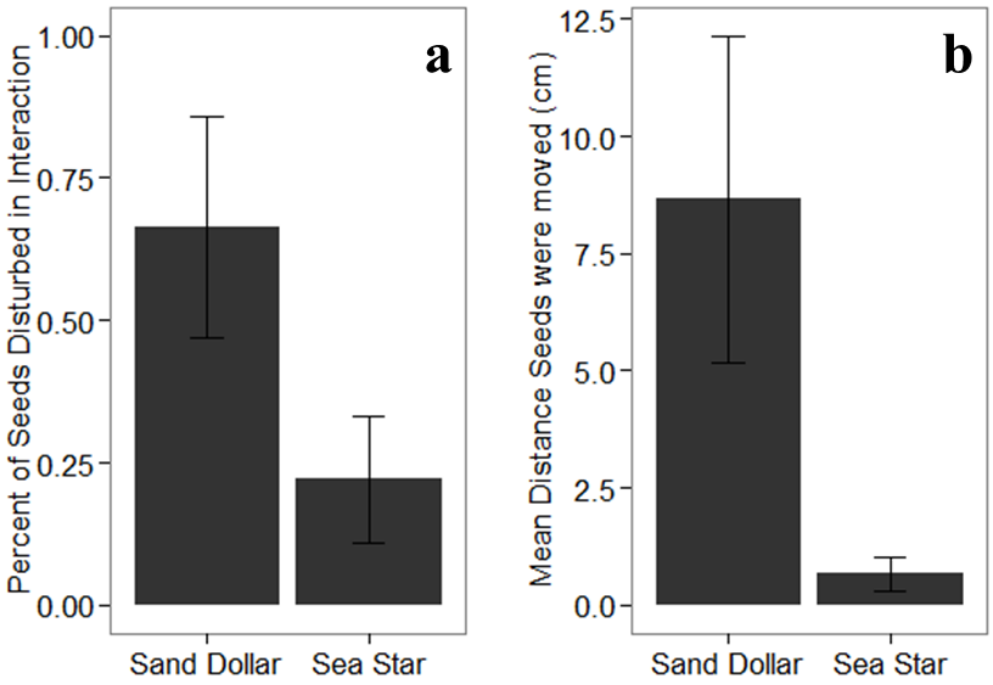

Figure S3. (a) The mean percentage of seeds disturbed (± SE) after being placed in the path of free roaming sand dollars (n = 3) and sea stars (n = 9). (b) The mean distance (± SE) free roaming sand dollars and sea stars moved seeds placed into their path.

Figure S4

Figure S4. A blue swimmer crab (*Portunus armatus*) burrowing into sediment on which seeds have recently settled (behind).

CHAPTER TWO SUPPLEMENTARY MATERIAL

Supplementary Tables

Table S1

Table S1. The species identified within the field of view from 78 hrs of time series photography conducted in the James and Chickahominy Rivers, Virginia, from August – September 2017. All animals moving into the field of view which could not be identified were categorized a "Unidentifiable."

Species	Scientific Name	Number of Observations
Juvenile Sunfish	*Lepomis* sp.	42
Spot	*Leiostomus xanthurus*	22
Blue Crab	*Callinectes sapidus*	17
Tessellated Darter	*Etheostoma olmstedi*	16
Bay Anchovy	*Anchoa mitchilli*	1
Blue Catfish	*Ictalurus furcatus*	1
Striped Bass	*Morone saxatilis*	1
American Eel	*Anguilla rostrata*	1
Unidentifiable	-	106

Table S2

Table S2. A generalized linear model of grazing intensity of 20 transplants after 24 hours and 1 week along transects at two locations over three separate trials in late summer 2016. Asterisks indicate the statistical significance of coefficients at $p < 0.05$ (*) or $p < 0.001$ (**).

Variable	**Coefficient**	**Std. Error**	**t-value**	**P - value**
Duration: 1 Week	9.3	1.7	-3.9	< 0.001**
Location: Chickahominy	2.6	1.8	4.0	0.11
Trial: Trial 2	0.37	1.5	1.6	0.01*
Trial: Trial 3	0.20	1.5	-2.6	< 0.001**
Duration*Location	2.6	2.0	-3.8	0.18
Intercept	0.14	1.6	1.3	< 0.001**

Table S3:

Table S3. Fauna identified within *Najas minor* meadows adjacent to the experimental area.

Habitat	Species	Number of Observations
Epifauna	*Lymnea* spp.	116
	Mytilopsis leucophaeata	115
	*Unidentified caddisfly**	31*
	Corophium sp.	18
	Mayfly Nymphs (*Ephemeroptera)*	10
	Gammarus spp.	3
	Lembos smithii	2
	Palamaentes pugio	2
	Syngnathus fuscus	1
	Unidentifiable Amphipods	6
	Unknown tubeworm	1
	Chironomidae larvae	> 100
Infauna	*Tubifex* sp.	5

* Unidentified caddisfly was observed at far higher frequency along the stems of *Najas minor* within the system.

Supplementary Figures

Figure S1:

Figure S1. Aluminum wire cages used to exclude or contain blue crabs during *in situ* caging studies. The plastic bottom of each cage (in blue) was inserted into the sediment at least 7 cm. One 50 cm L, 5 cm W PVC stake and one 50 cm rebar stake were placed within plastic ties on either side of the bucket to anchor it in place.

Figure S2:

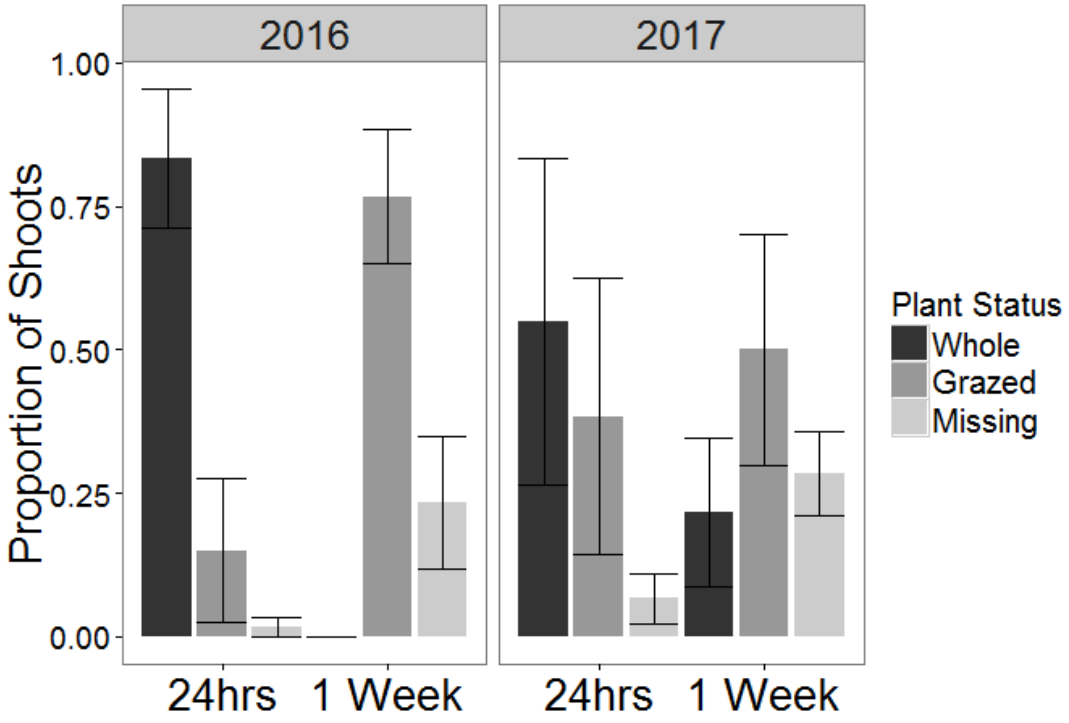

Figure S2. The proportion of shoots (± SE, n = 20) consumed within 24 hours and after one week along transects (n = 3) at the mouth of the Chickahominy River in 2016 and 2017.

Figure S3:

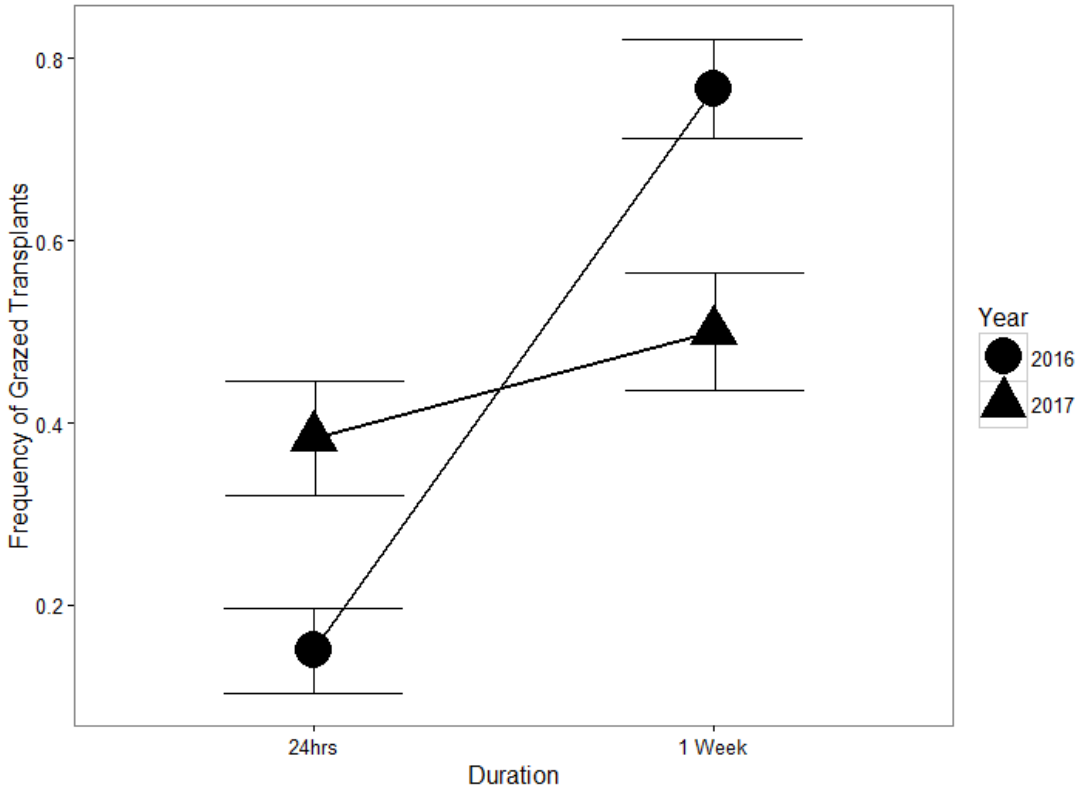

Figure S3. The mean frequency (± SE) of grazed transplants (n = 3 transects) observed in late summer of 2016 and 2017.

Figure S4:

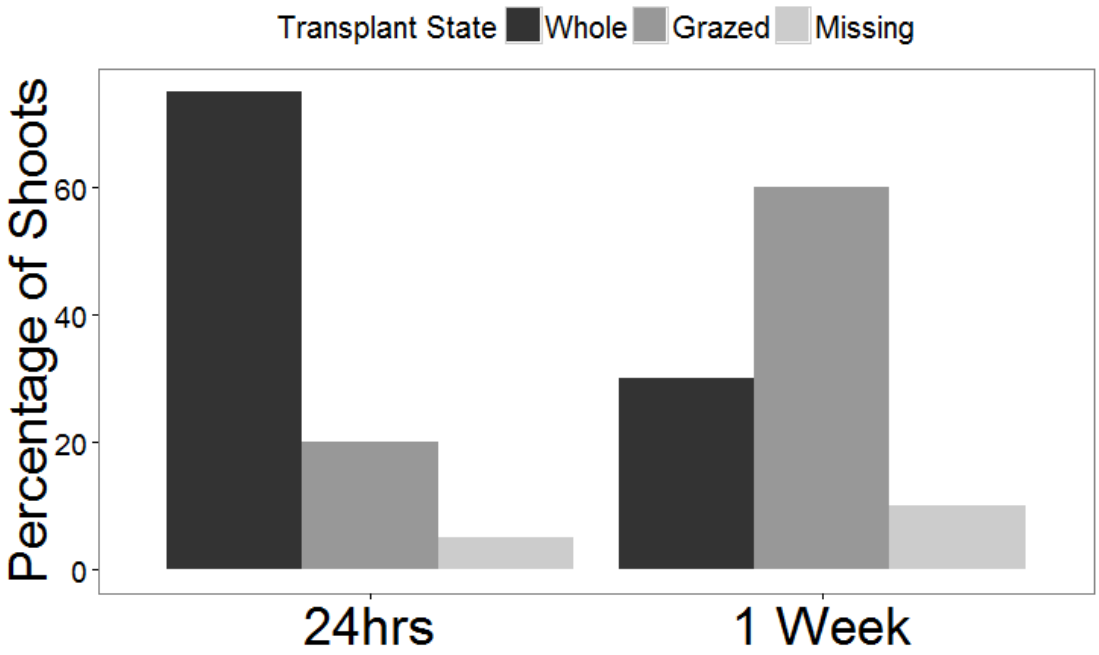

Figure S4. The percentage of *Vallisneria americana* transplants (n = 20) clipped within a *Najas minor* meadow adjacent to transect lines in summer 2016.

Figure S5

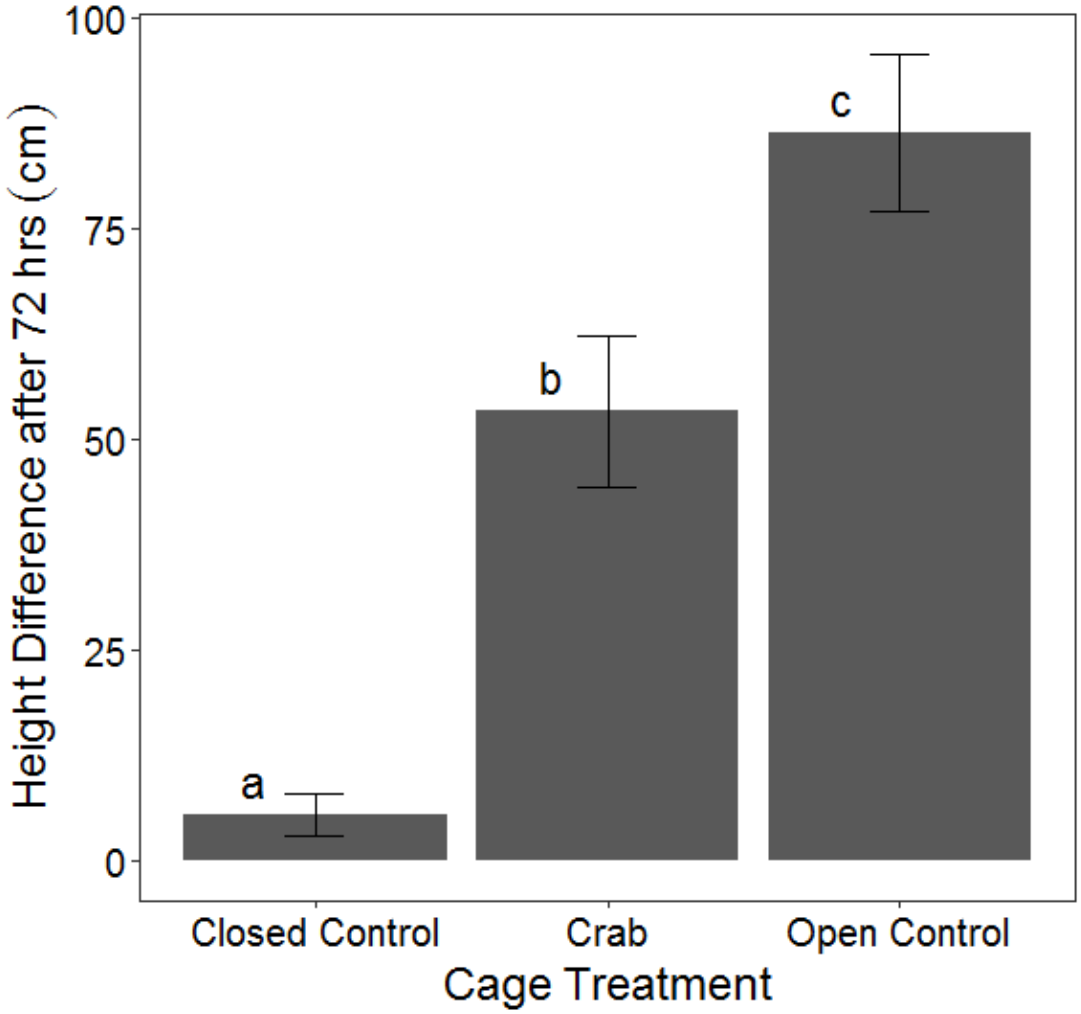

Figure S5. The mean difference in leaf height per cage (± SE) for *V. americana* shoots planted in cages (n = 31) without crabs (Closed Control), with crabs (Crab), and outside any cage (Open Control). Letters indicate significant differences ($p < 0.05$).

Figure S6

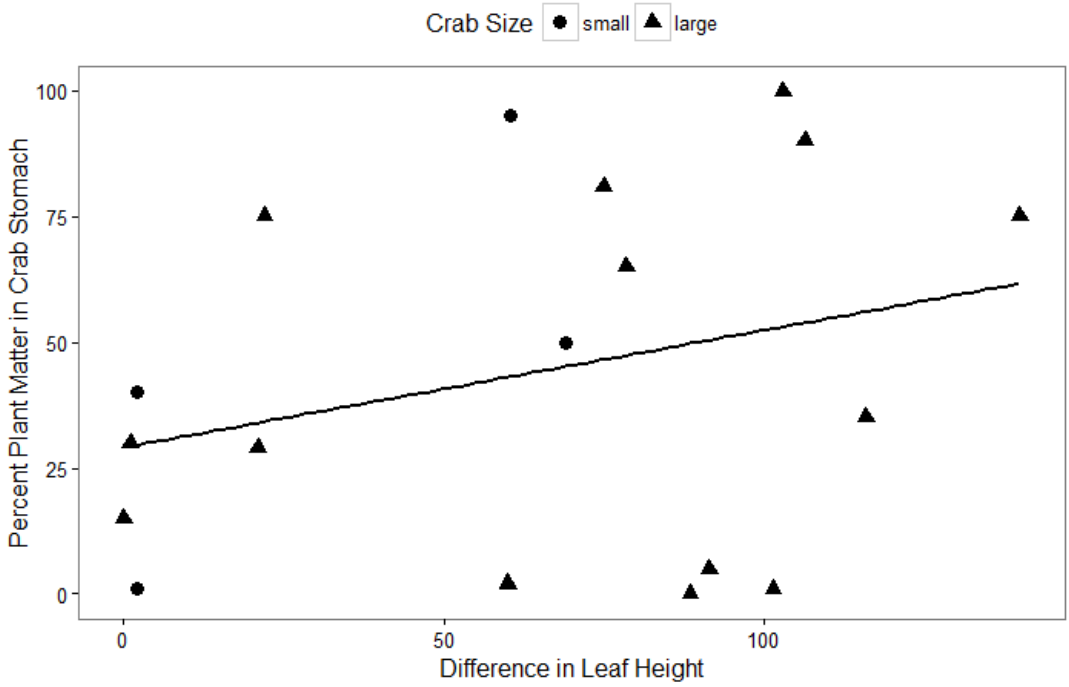

Figure S6. A scatterplot of the difference in transplant leaf height and the percent of plant matter in crab stomachs for each *in situ* caging trial for which the crab could be recovered with a non-significant trend line ($p = 0.1$). Small crabs (CW < 8 cm) are displayed with closed circles and large crabs ($\geq$ 8 cm) are displayed with closed triangles.

Figure S7

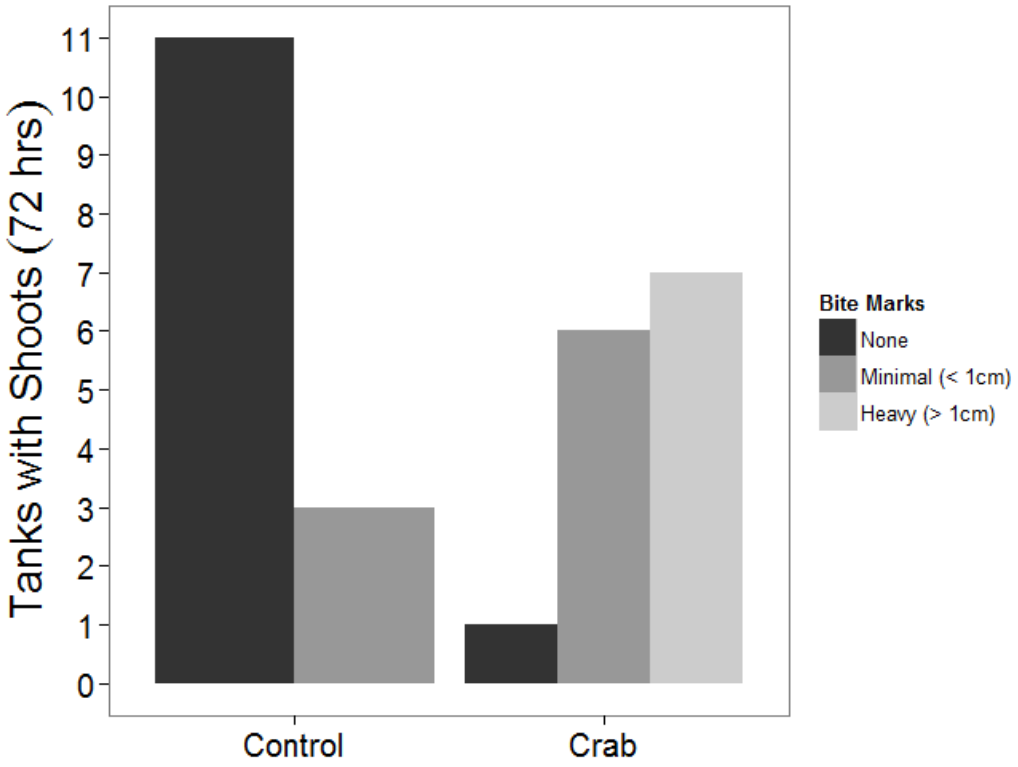

Figure S7. The frequency of bite mark categories observed on *V. americana* shoots from tanks with and without blue crabs.

Figure S8:

Figure S8. A juvenile blue crab clipping the leaves of a *Hydrilla verticillata* propagule in a preliminary feeding experiment. Blue crabs in these preliminary trials also consumed *Najas minor* transplants (pictured in the background), a finding stomach contents of wild-caught blue crabs corroborates.

Figure S9

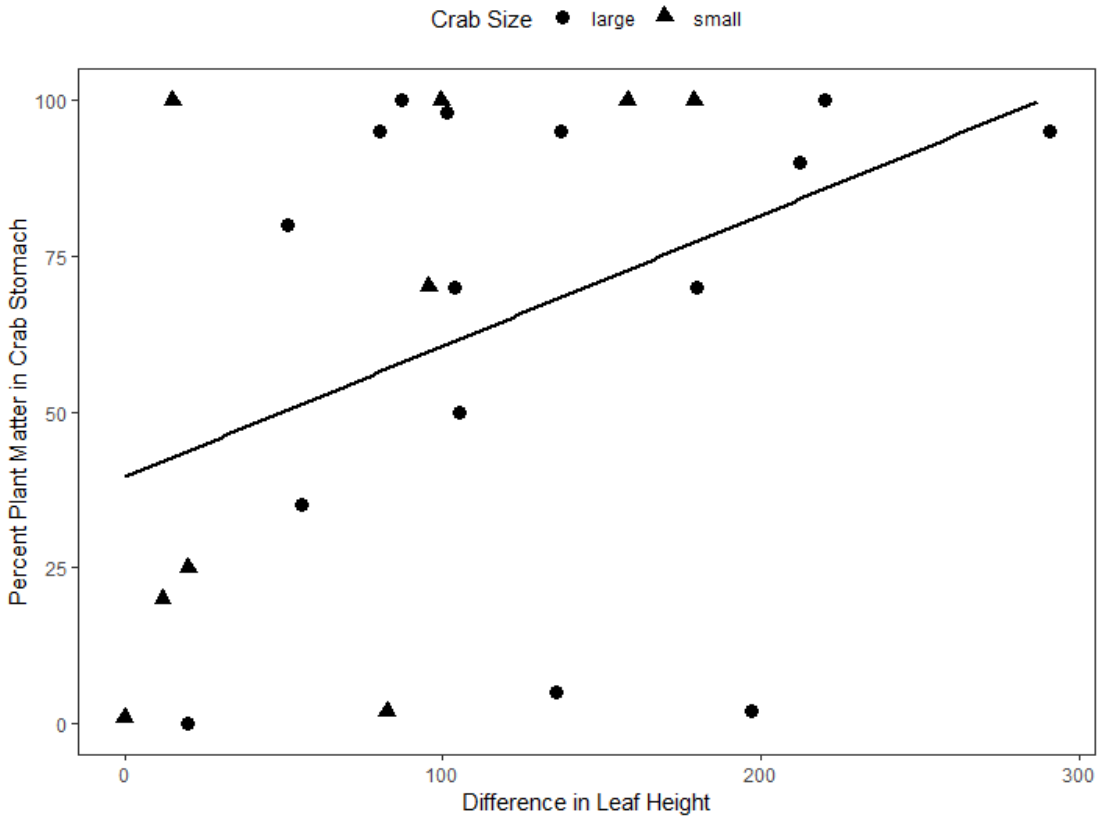

Figure S9. A scatterplot of the difference in total leaf height and the percent of plant matter in crab stomachs for each tank during blue crab preference experiments with a non-significant trend line ($p = 0.05$). Small crabs (CW < 8 cm) are displayed with closed circles and large crabs ($\geq$ 8 cm) are displayed with closed triangles.

Figure S10

Figure S10. Floating fragments of *Vallisneria americana* (circled in blue) and *Hydrilla verticillata* (circled in red) clipped by *Callinectes sapidus* during a preliminary feeding trial. Dark matter on the sand at the bottom of the tank were clipped leaves of *H. verticillata*.

Figure S11:

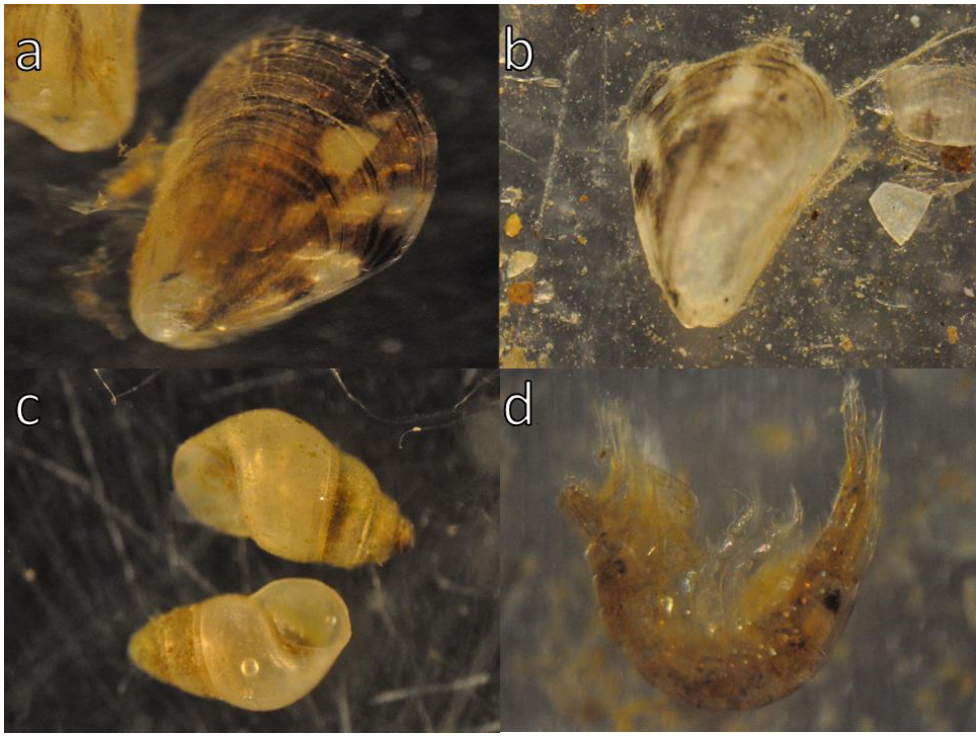

Figure S11. Prey in the stomachs of blue crabs collected within a meadow of non-native vegetation : (a) A shell of *Mytilopsis leucophaeata* from an epifaunal grab bag sample; (b) a magnified imagine of a *M. leucophaeata* shell found in the stomach of a blue crab collected from the Chickahominy River; (c) the shells of *Lymnea spp.* gastropods collected from Chickahominy River and identified in blue crab stomachs; and (d) a *Corophium spp.* amphipod found in the stomach of a blue crab and within epifaunal samples from non-native vegetation in the Chickahominy River.

Figure S12:

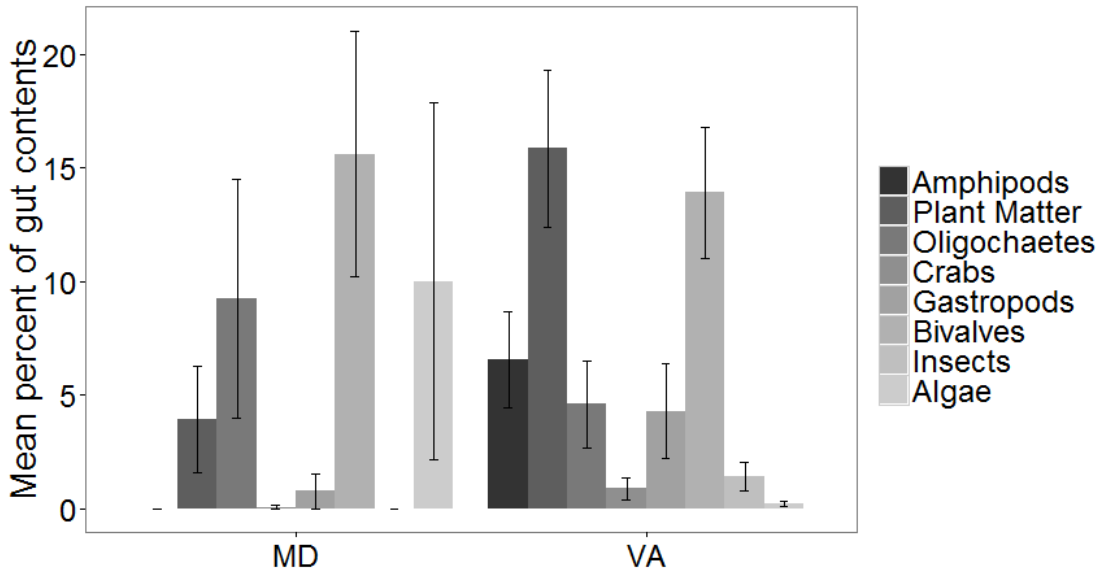

Figure S12. The mean percentage of identifiable food items in the guts of blue crabs collected in the lower Chesapeake Bay (VA, n = 52) and upper Chesapeake Bay (MD, n = 13). To evaluate whether the diets of blue crabs differed significantly in tidal freshwater areas with established *V. americana* populations to areas where *V. americana* has not recovered, the stomach contents of blue crabs captured at locations with *V. americana* in the upper Chesapeake Bay (Maryland) were compared to the stomach contents of blue crabs in the lower Chesapeake Bay (Virginia) where *V. americana* populations have not recovered. Maryland crabs were caught within additional seine surveys at two locations in the Elk River and one within the Susquehanna Flats; both locations of extensive SAV meadows with abundant *V. americana* populations. Plant matter was found in blue crab stomachs from both locations, but a considerable range in plant and overall diet may exist between locations. Due to low sample sizes caught in Maryland, the diet of blue crabs caught between these two locations in seine surveys were not presented as formal comparisons. Future research should explore potential factors, e.g. blue crab diet differences, alternative grazer communities or dynamics, or adjacent *V. americana* donor meadows, allowing *V. americana* populations to overcome grazing in Maryland, but not in Virginia.

Figure S13:

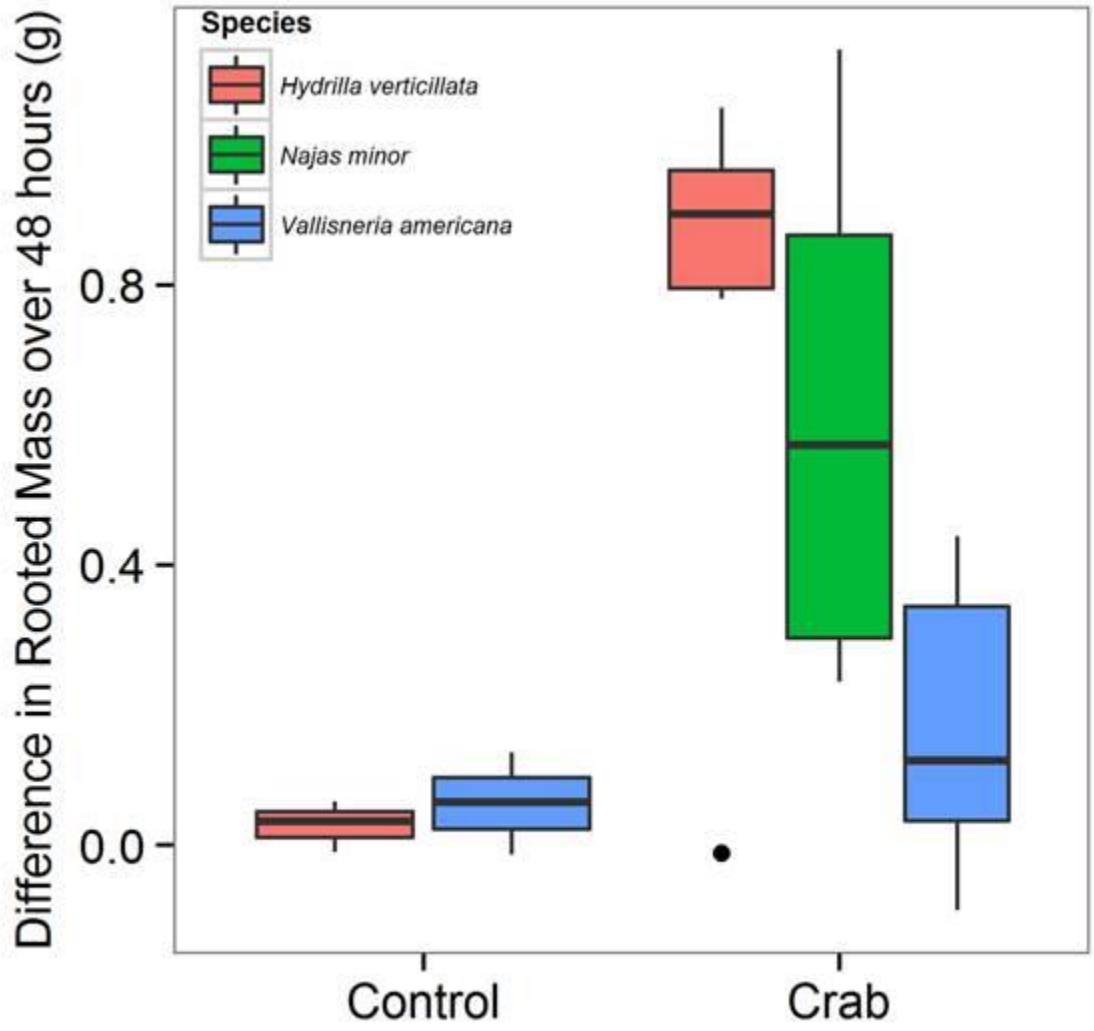

Figure S13. The difference in biomass of SAV species left with a single blue crab for 72 hours in a tank environment. Plants were dried in a salad spinner according to a fixed protocol before weighing. This preliminary study was not conducted in a temperature controlled water bath, so was not included in the main study as temperature fluctuations could impact blue crab behavior. In addition, blue crab clipping, consumption, and damage to plant matter made recovery and drying of all pieces of vegetation after blue crab exposure difficult. Last, we did not have sufficient *N. minor* to plant in control tanks. As a result, any statistical comparison for this species would be impossible. We include this figure simply to demonstrate blue crabs damaged, clipped, or consumed all three species of SAV featured in the study. Boxes and whiskers indicate data quartiles and dark bars across boxes indicate data medians.

Video S1:

https://drive.google.com/file/d/13HF_nrLXk-nqvyR78dHIv5EukE8BwSHw/view?usp=sharing

Video S1. An uploaded video of a blue crab consuming epiphytes off the leaves of *Zostera marina* in the lower Chesapeake Bay, VA. This consumption behavior could potentially damage or tear leaves of SAV and is worthy of further investigation.

CHAPTER THREE SUPPLEMENTARY MATERIAL

Supplementary Tables

Table S1. A tabulated summary of the methods.

Project	Method	Sites	Years	Sampling Period	Metrics Measured
Natural Seedling Establishment	Survey	Bena Allens Island Sandy Point Goodwin Island	2016 2017 2018	Annually	Number and height of seedlings present
Potential Seed Limitation	Manipulation	Bena Allens Island Sandy Point Goodwin Island	2016 2017 2018	Annually	Number and height of seedlings present
Survey of Seedling Survival amongst Adult Shoots	Survey	Bena Allens Island Sandy Point Goodwin Island	2017	Monthly (April - October)	- # of Seedlings Surviving - Density, height, and bottom cover of adult shoots of both *Z. marina* and *R. maritima*
Seedling Competition	Manipulation	Bena	2017	Weekly (May - November)	Seedling Survival
Transplant Gardens: $1m^2$	Manipulation	York River	2016 2017 2018	Monthly (May - October)	Percent Bottom Cover
Transplant Gardens: $4m^2$	Manipulation	York River Spider Crab Bay	2017 2018	Monthly (May - October)	Percent Bottom Cover

Table S2. The statistics used to evaluate each objective.

Experimental Method	Response Variable	Sample Unit	Fixed Variables	Random Variables	Statistical Method
Survey of seedling establishment	Number of Seedlings	Core	• Location • Year	-	Generalized linear model (Poisson Distribution)
Seed addition Experiment	Number of Seedlings	Core	• Treatment (Natural or added seeds)	• Location • Year • Block	Generalized linear-mixed effects model (Poisson Distribution)
Seedling and Adult Height Comparison	Height of seedlings	Seedlings and haphazardly selected adult shoots	• Life Stage	• Year • Location	Mixed effects model (Gaussian Distribution)
Survey of seedlings	# of seedlings surviving m^{-2}	1 m^2 of surveyed area	• *Z. marina* leaf area index (LAI) • *R. maritima* LAI	• Location • Month	First-order autoregressive model (Gaussian Distribution)
Seedling Competition Experiment	Seedling Survival	Tagged seedling	• Competition Treatment (with/ without neighbor shoots)	-	Kaplan-Meier Curves and log-rank test
Transplant Garden Experiments	Percent Bottom Cover	1 m^2 plot	• Reproductive Treatment (4 levels)	• Year (2016-2018) • Month (May-Oct) • Block (1-8)	First-order autoregressive model (Gaussian Distribution)
Transplant Garden Experiments	Percent Bottom Cover	4 m^2 plot	• Reproductive Treatment (4 levels)	• Year (2017-2018) • Month (May-Oct) • Block(1-4)	First-order autoregressive model (Gaussian Distribution)

Table S3. Analysis of deviance table for the generalized linear model of natural seedling establishment across four meadows in the lower York River in late May/early June 2016, 2017, and 2018.

Term	DF	Deviance	Residual DF	Residual Deviance	P value
Null			139	289.15	
Year	2	33.4	137	255.78	< 0.001
Location	3	15.2	134	240.61	0.002
Year*Location	6	85.4	128	155.18	< 0.001

Table S4. The mean height of seedlings (± SE) and randomly selected adult shoots (± SE).

Location	Mean Seedling Height (cm)	Mean Adult Shoot Height (cm)	Difference in Mean Heights (cm)	Mean Seedling Shoot Number
AI	18 (0.9)	58 (2.7)	40	1.1 (0.085)
GI	14 (0.5)	23 (3.3)	9	1.2 (0.054)
BE	14 (1.1)	51 (2.7(	37	1.0 (0.021)
SP	25 (1.0)	46 (3.2)	21	1.9 (0.18)

Table S5. The parameters from a generalized linear mixed effects model of seedling establishment between plots receiving 1000 additional seeds m^{-2} relative to control plots with only natural seedling establishment at four locations over three years in the lower Chesapeake Bay.

Fixed Effects	Term	Estimate (±SE)	Z value	P value
	Seed Application	2.0 (1.1)	6.7	< 0.001
	Intercept	0.61 (1.6)	-1.1	0.27

Random Effects	Groups	Variance
	Location:Year	0.67
	Year	0.41

Table S6. The parameters from a generalized linear mixed effects model of seedling establishment between plots receiving 1000 additional seeds m^{-2} relative to control plots with only natural seedling establishment at four locations over three years in the lower Chesapeake Bay.

Fixed Effects	Term	Estimate (±SE)	t value	P value
	Intercept	0.32 (0.13)	2.2	0.02
	Z. marina LAI	-0.090 (0.026)	-4.8	< 0.001
	R. maritima LAI	0.088(0.024)	2.7	< 0.001

Random Effects	Groups	Variance
	Location:Month	< 0.001
	Month	0.31
	Residual	0.22

Table S7. Results from an analysis of deviance of percent bottom cover between treatments with and without sexual reproduction in experimental transplant plots in the lower York River and Spider Crab Bay from May – October in 2017 and 2018.

	Numerator DF	**Denominator DF**	***F***	***p***
Intercept	1	1005	34	< 0.001
Reproductive Treatment	2	94	10.0	< 0.001
Location	1	2006	40	< 0.001
Treatment*Location	2	1005	13	< 0.001

Supplementary Figures

Figure S1.

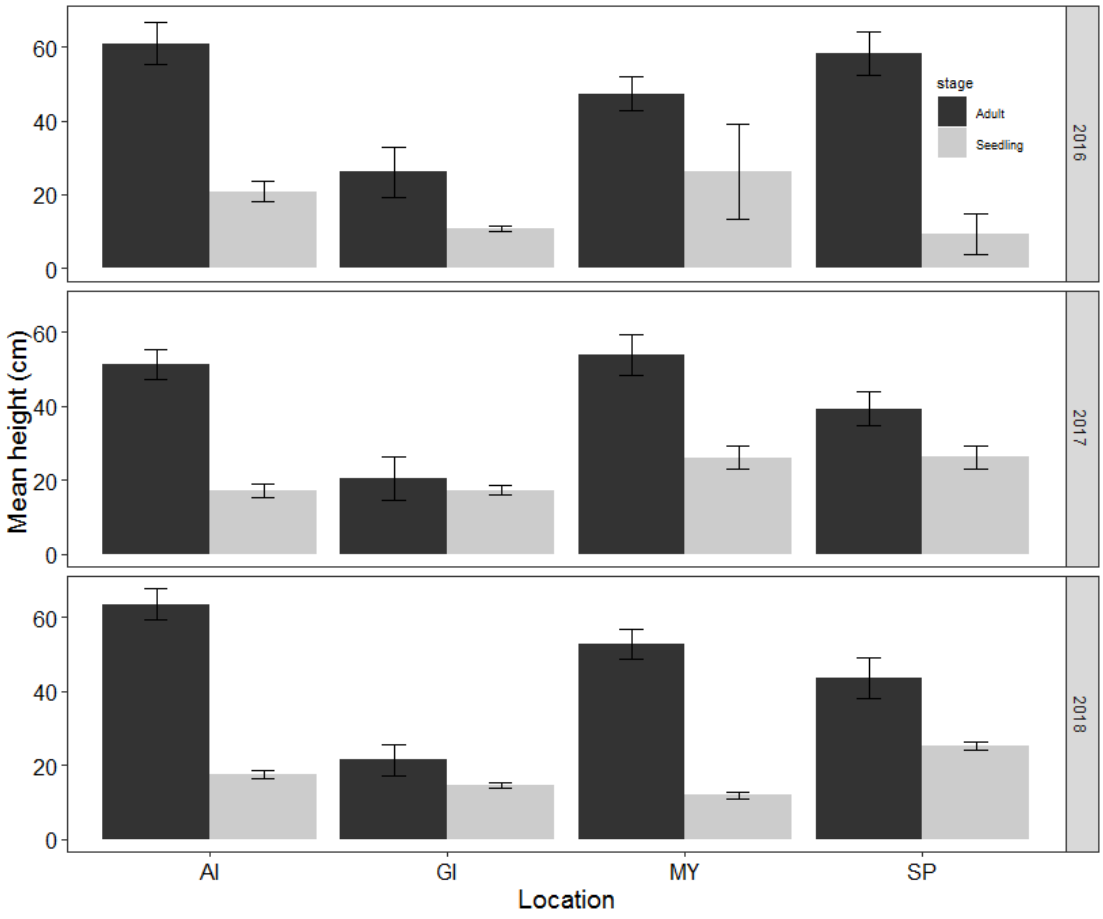

Figure S1. The mean height (± SE) of seedlings and haphazardly selected adult shoots at each location

Figure S2.

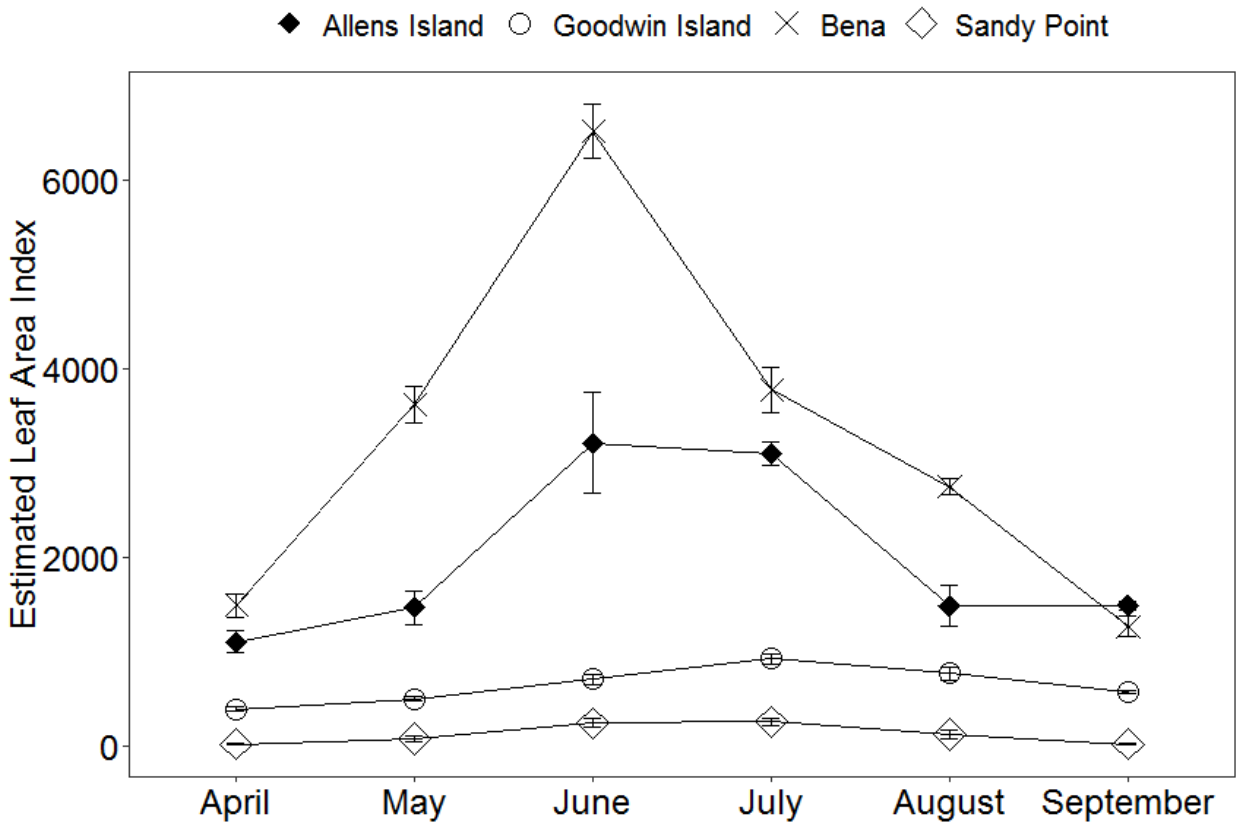

Figure S2. The mean leaf area index (± SE, n = 4) of *Z. marina* measured at each location in 2017.

Figure S3.

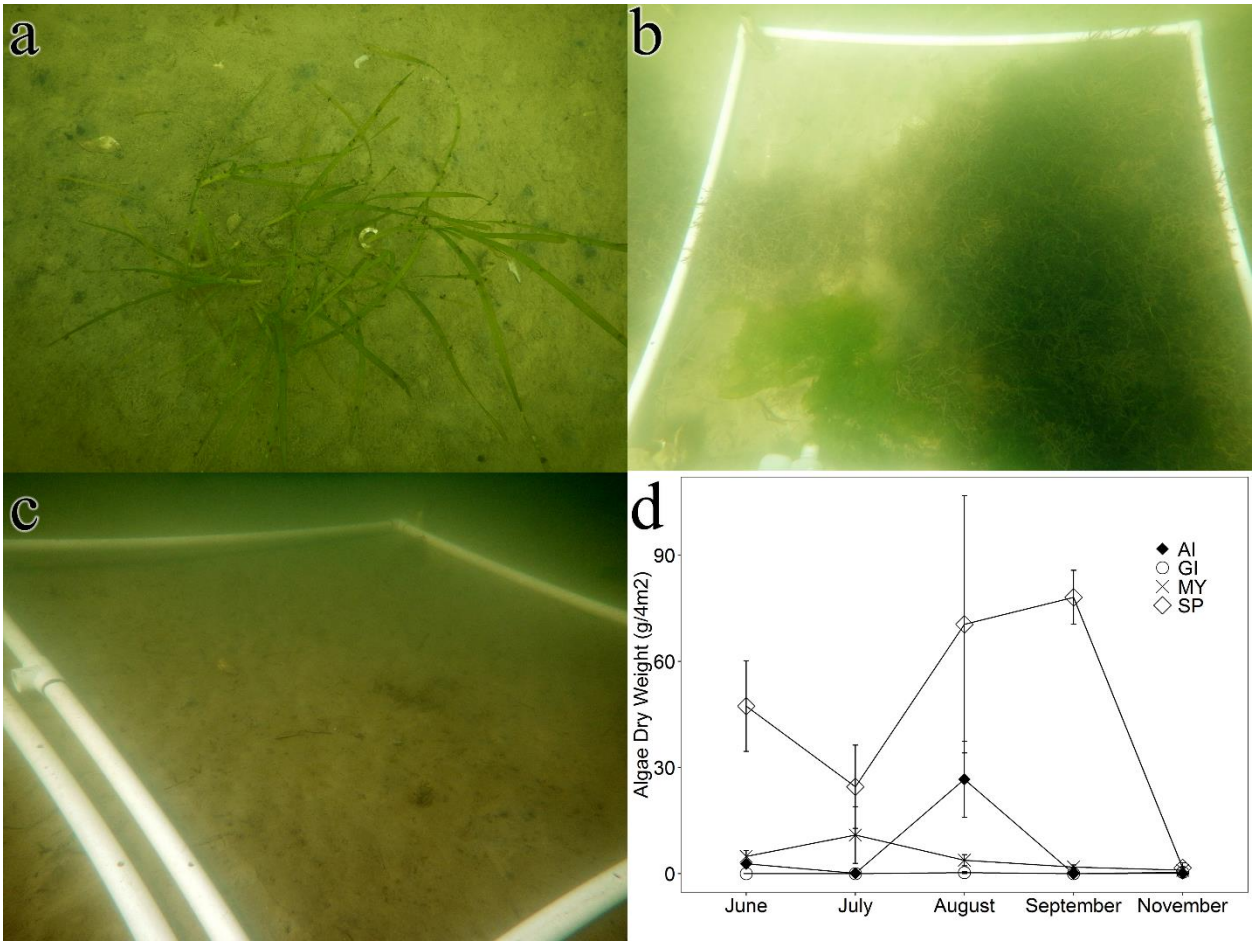

Figure S3. The impact of algal mats on seagrass at Sandy Point: a. A large seedling tagged with a 19mm steel lock washer in April 2017; b. An example of a macroalgae mat covering plots at Sandy Point in the summer of 2017; c. A now bare plot in November 2017; d. the dry weight of algae collected and dried from four, 1 m^2 quadrats haphazardly thrown at each location.

Figure S4.

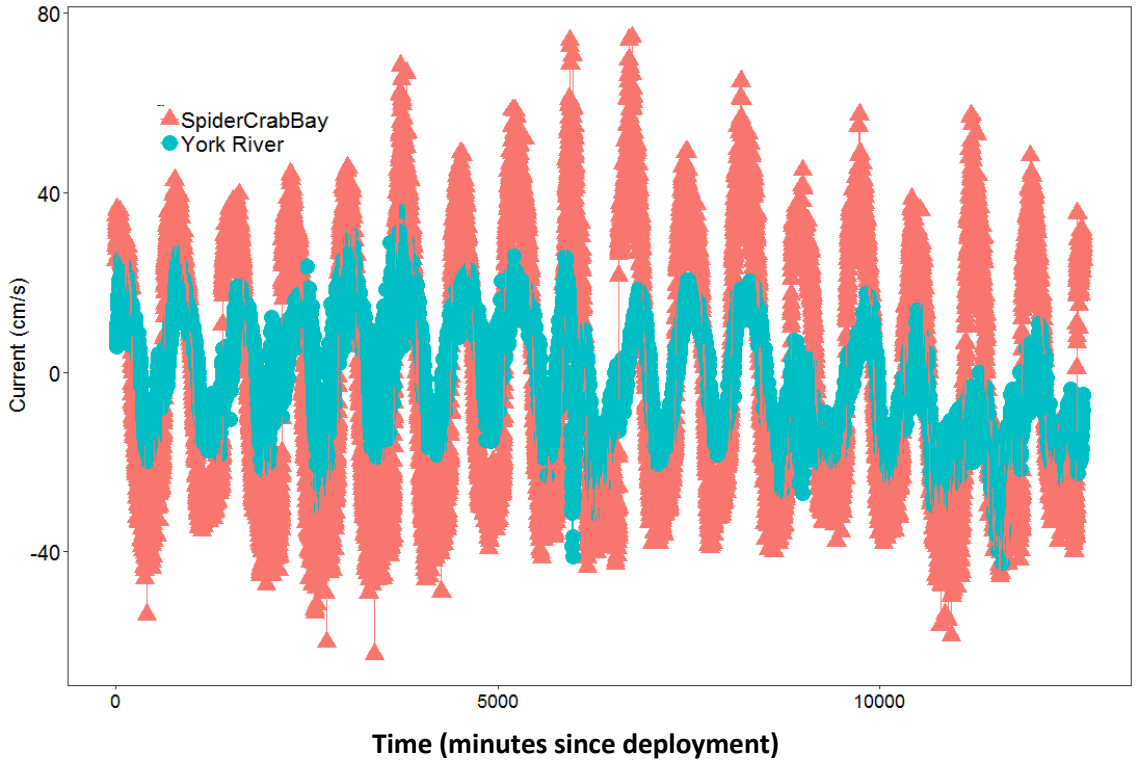

Figure S4. The velocity of water recorded by tilt current meters adjacent to the experimental transplant plots in the York River, VA near Goodwin Neck and in Spider Crab Bay on the Delmarva Peninsula, VA over nine days.

Supplemental Canopy Light Survey

To determine if established shoots surrounding seedlings alter light availability to seedlings at each location, a survey of light penetration was conducted with HOBO light sensors adjacent to each plot with tagged seedlings. Three HOBO© light and temperature sensors were deployed 10cm above the sediment surface simultaneously for three days recording temperature (^{o}C) and light (lumens) every minute at each location. Two sensors were haphazardly labelled and placed amongst established shoots of *Z. marina* and *R. maritima* and one sensor was placed within a ~ 0.07 m^2 bare space. All sensors were deployed within ~2 m of the experimental plot. The areas selected for sensor deployment were also evaluated to ensure the canopy height was representative of the adjacent experimental plots and the density of shoots and heights of three shoots within 0.07 m^2 of the sensor was recorded to estimate a leaf area index. These light sensors measured light in lumens ft^{-2} which, although not directly measuring photosynthetically active radiation, have been used to estimate relative light availability (Wall et al. 2011, Long et al. 2012). For the purpose of this study, these comparisons should be viewed as rough estimates of the relative light available in each environment.

A Welch's t-test for unequal variance was used to test if the amount of light reaching light sensors beneath seagrass was lower than the light reaching sensors in bare sediment. One of the sensors placed in seagrass at each location was randomly selected to be compared to the control sensor in bare sediment. A correlation was used to test for any relationship between the LAI of surrounding vegetation and the amount of light reaching the sensor. The amount of light the sensor measured was $\log_{10}$ transformed to meet model assumptions.

Light sensors placed within seagrass canopies recorded significantly less light than sensors placed in bare sediment ($F_{1,10495} = 1004$, $p < 0.001$). The LAI of vegetation surrounding a sensor was negatively related to the average light reaching the sensor ($r = -0.73$, $t = -2.6$, $p = 0.04$).

Literature Cited

Long MH, Rheuban JE, Berg P, Zieman JC (2012) A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. Limnol. Oceanogr.: Methods 10: 416 – 424. doi: 10.4319/lom.2012.10.416

Wall CC, Peterson BJ, Gobler CJ (2011) The growth of estuarine resources (*Zostera marina, Mercenaria mecenaria, Crassostrea virginica, Argopecten irradians, Cyprinodon variegatus*) in response to nutrient loading and enhanced suspension feeding by adult shellfish. Estuar Coasts 34: 1262 – 1277. doi: 10.1007/s12237-011-9377-7

Figure S5.

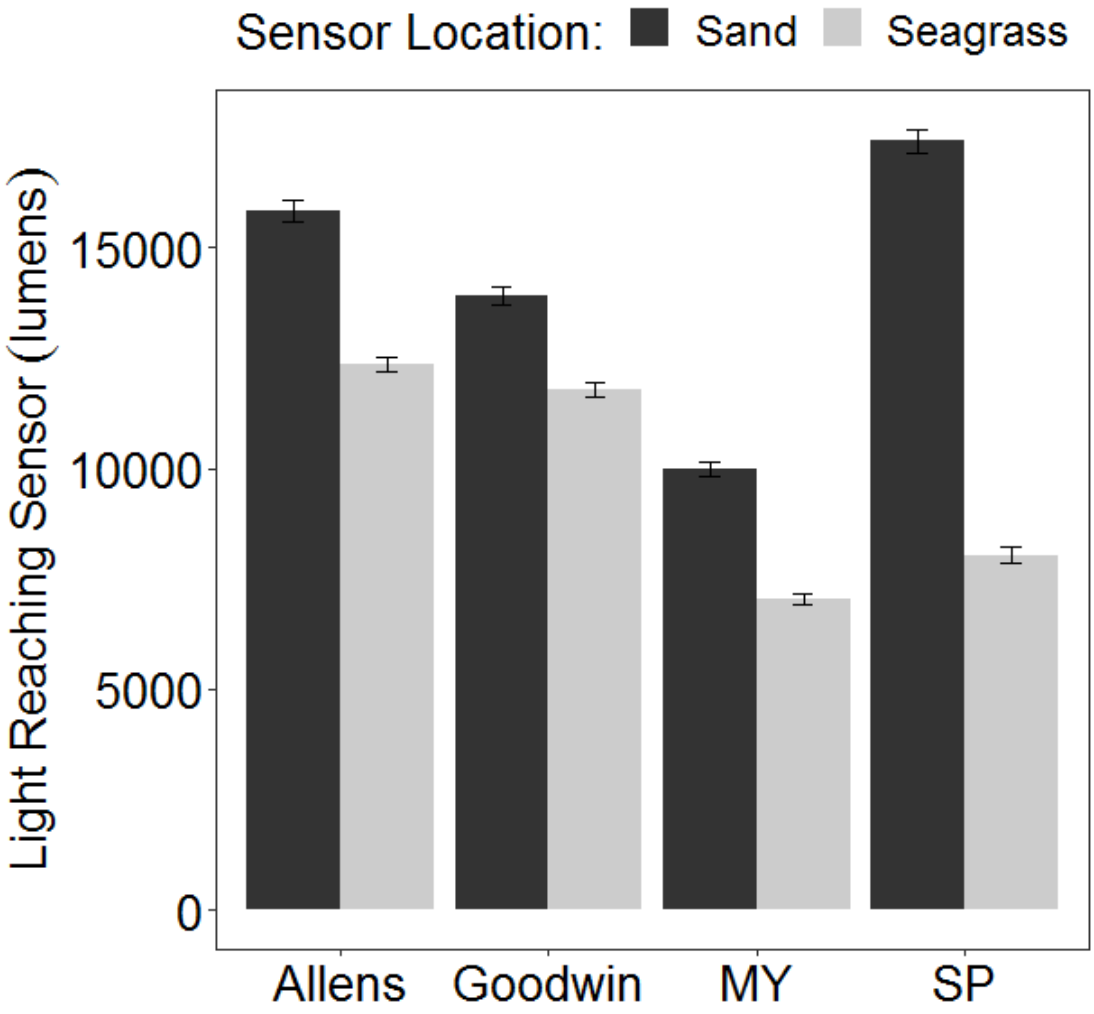

Figure S5. The mean lumens (± SE) reaching HOBO sensors placed 10cm from the sediment bottom in seagrass or sand. Sensors placed in seagrass measured significantly less light (β= -6.3 ± 1.0, t = -29, $p < 0.001$) than sensors placed in sand.

Figure S6

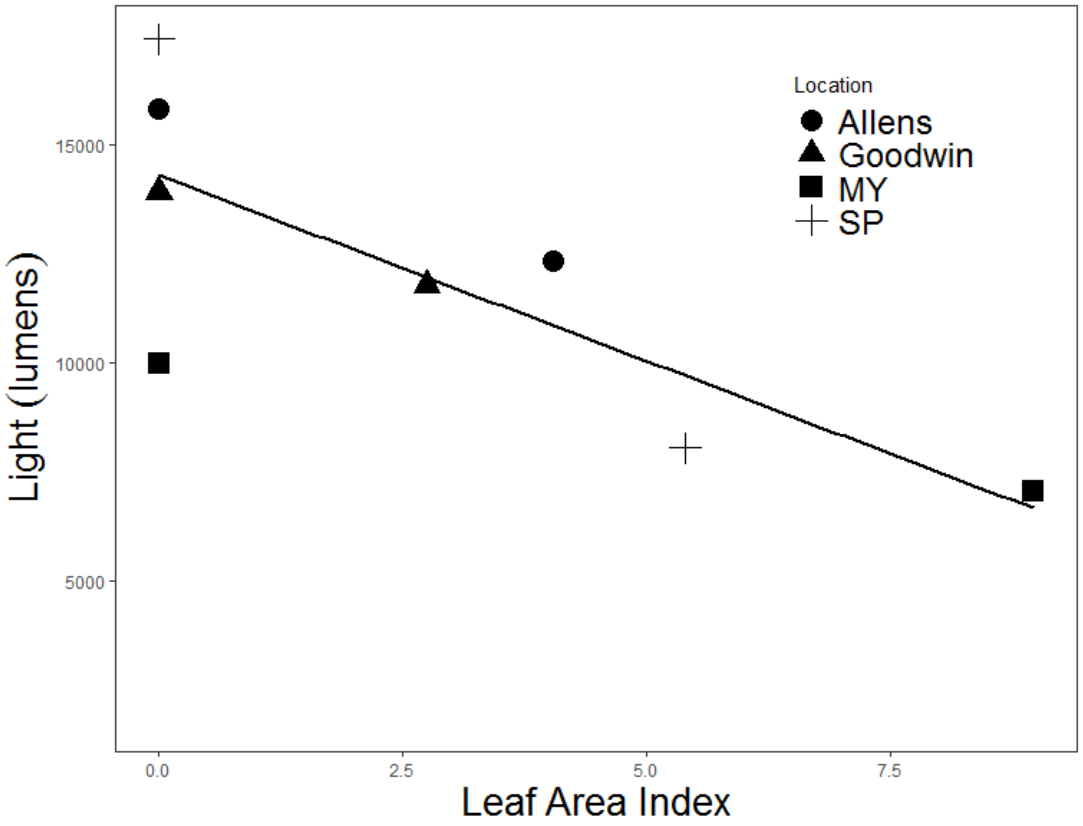

Figure S6. The mean light (lumens/ft^2) reaching sensors with increasing mean leaf area index measured at four locations in the lower York River, VA.

Figure S7.

Figure S7. The disparity in size of seedlings taken from one random core at Goodwin Island in early June 2018.

Figure S8:

Figure S8. The disparity in size of seedlings found in one random core from Sandy Point in early June 2018.